

Recension of the Mexican species of *Hymenostephium* (Asteraceae: Heliantheae)

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ABSTRACT

Eight species of **Hymenostephium** are recognized as occurring in Mexico: a widespread, highly variable, **H. cordatum** (including *H. brandegianum*); **H. gracillimum**, localized, shoreline endemic of Oaxaca and Chiapas; **H. hintonii**, montane endemic of Michoacan and Guerrero; **H. superaxillare**, localized taxon from southern Chihuahua and closely adjacent Durango; **H. tenuis**, widespread montane taxon occurring along the Pacific slopes from Nayarit to Chiapas; **H. uniseratum**, montane areas of central Mexico; **H. websteri**, very localized endemic of southern Nayarit and closely adjacent Jalisco; and **H. woronowii**, a very localized endemic of Uruapan, Michoacan and vicinity. Keys to the taxa are provided, along with maps showing their distribution. Published on-line: www.phytologia.org *Phytologia* 95(1): 1-9 (Feb. 1, 2013).

KEY WORDS: Asteraceae, Heliantheae, Mexico, *Hymenostephium*, *Viguiera*

The present contribution was stimulated by the recent account of derived lineages within the tribe Heliantheae (Schilling and Panero 2002; 2011, as cited below). The treatment follows the format adopted for my on-going Comps of Mexico (Phytologia Memoirs 1, 6, 12, 14, 15; etc.).

HYMENOSTEPHIUM Benth.

Garcilassa Poepp.

Haplocalymma Blake

Viguiera sect. *Diplostichis*

Annual or perennial herbs. Leaves mostly opposite, without resin dots upon surfaces; blades ovate, elliptic to lanceolate, the blades variously serrate; petioles present, rarely not. Heads campanulate, rarely cylindric, relatively small, mostly in terminal cymose panicles or, less often, single on elongate ultimate peduncles. Involucral bracts 1-3 seriate, few to numerous. Receptacles convex, rarely conic, paleate. Ray florets 5-21, neuter, sterile, rarely absent; ligules yellow, rarely white. Disc florets 10-numerous; corollas yellow. Achenes ovoid, glabrous or appressed-pubescent; pappus of two-awned scales, between these 2-4 short scales, or pappus absent. Base chromosome numbers, $x = 12$ and 17 .

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 Turner, B.L., M. Powell and R.M. King. 1962. Chromosome numbers in the Compositae. VI. Additional Mexican and Guatemalan species. *Rhodora* 64: 251-271.

Blake (1918, p. 6), in his monographic treatment of **Viguiera**, prepared a key to the latter and presumed allies; in this he recognized **Hymenostephium** as a distinct genus of several species, most of its taxa having a pappus of two awns, between these several membranous scales. Schilling and Panero (2002, 2010), using DNA data, retained the genus, noting that it contained ca 26 species distributed from Mexico to Central and South America. In my forthcoming treatment of **Hymenostephium** for Mexico, I recognize 7 species, as follows:

Key to species

1. Tap-rooted annuals, or erect, simple-stemmed, perennials, 20-80 cm high.....(4)
1. Suffrutescent herbs, or recumbent shrubs 1-4 m high.....(2)
2. Heads relatively small, 4-5 mm high, 2-3 mm wide (rays excluded); disc florets 5-10; leaves glabrous, or nearly so; oak forests, Mic, Gue.....**H. hintonii**
2. Heads relatively large, 4-7 mm high, 4-10 mm wide; disc florets mostly 12 or more; leaves variously pubescent.....(3)
3. Involucres 8-10 mm across; ray florets 11-13; s Chi, n Sin.....**H. superaxillare**
3. Involucres 4-6 mm across; ray florets 5-8; widespread.....**H. cordatum**
- 4(1). Perennial herbs 20-80 cm high, arising from a corm-like base; achenes pubescent, epappose; Nay, Jal.....**H. websteri**
4. Annual herbs 20-80 cm high, arising from slender tap-roots; achenes otherwise.....(5)
5. Outer involucral bracts mostly in 1-2 series, grading into the inner bracts.....(7)
5. Outer involucral bracts elliptic-ovate, exactly 5, in a single whorl.....(6)
6. Leaves mostly 1-2 cm long, 0.2-0.8 cm wide; involucres ca 2 mm high; Mic.....**H. woronowii**
6. Leaves mostly 3-6 cm long, 1-4 cm wide; involucres 4-5 mm high; Mex, Mor, Gue....**H. uniseratum**
7. Involucres 2-4 mm high; achenes glabrous; Mic**H. woronowii**
7. Involucres 4-12 mm high; achenes pubescent.....(8)
8. Leaves sessile or nearly.....**H. tenuis**
8. Leaves with well-defined petioles 3-15 mm long; Pacific shore lines, se Oax, sw Cps.**H. gracillimum**

HYMENOSTEPHIUM CORDATUM (Hook. & Arn.) Blake, J. Bot. 53: 268. 1915.

Aspilia hispida Brandege

Gymnolomia ehrenbergiana Klatt

Gymnolomia guatemalensis (Rob. & Greenm.) Blake

Gymnolomia microcephala Less.

Gymnolomia microcephala var. *guatemalensis* (Rob. & Greenm.) Rob. & Greenm.

Gymnolomia patens A. Gray

Gymnolomia patens var. *abbreviata* Rob. & Greenm.

Gymnolomia patens var. *brachypoda* Rob. & Greenm.

Gymnolomia patens var. *guatemalensis* Rob. & Greenm.

Gymnolomia patens var. *macrophylla* Rob. & Greenm.

Hymenostephium brandegei (Blake) Schill. & Panero

Hymenostephium mexicanum Benth.

Hymenostephium pilosulum Blake

Montanoa thomasii Klatt

Viguiera brandegei Blake

Viguiera cordata (Hook. & Arn.) D'Arcy

Wedelia cordata Hook. & Arn.

Mostly Gulf and Pacific slopes, Nue, Sin, Dur, San, Hid, Nay, Jal, Col, Mic, Mex, Pue, Ver, Gue, Oax, Cps and Guatemala southwards, tropical deciduous and evergreen montane forests, 600-2100 m; Aug-Jan. **Map 1**

Perennial suffruticose herbs 1-3 m high or clambering, weak-stemmed, shrubs or vines, often to 5 m high when draped upon small trees. **Leaves** mostly opposite, sometimes, however, markedly alternate along upper stems, very variable as to size, shape and vestiture, but mostly broadly ovate, 3-nervate, 5-10 cm long, 2-6 cm wide, the margins dentate; petioles mostly 1-2 cm long. **Capitulescence** a terminal or axillary aggregation of 3-8 heads, the peduncles 1-8 cm long. **Heads** radiate, mostly narrowly campanulate, arranged in loose or congested, terminal corymbs, the ultimate peduncles quite short or rarely up to 8 cm long; involucre narrowly or broadly campanulate, 2-3 seriate, 4-6 mm high and as wide, the bracts graduate to subequal, usually blackish. **Receptacle** convex, paleate. **Ray florets** 5-8, neuter, the ligules yellow, 5-15 mm long. **Disc florets yellow**, very variable in number, 10-numerous. **Achenes** 2-3 mm long, glabrous or pubescent, epappose or a few vestigial scales. **Chromosome numbers**, $n = 34$ and 46 , based on $x = 17$ (Strother & Panero 2001).

This is an exceedingly variable species, especially in habit, foliage, vestiture, capitulescence, involucre characters and chromosome numbers. Numerous specific names have been proposed for the many forms and populations, but I think the complex is so variable as to preclude even meaningful varietal breakdown. I can, however, discern a broad morphological assemblage along the Pacific slopes from Sin to Oax that has smaller heads with mostly greenish involucre bracts and generally smaller rays, but such forms intergrade completely with the more typical forms which have larger heads (involucre 4-8 mm wide), longer rays (mostly 8-15 mm long) and generally broader, more pubescent leaves.

I have retained the anomalous **H. hintonii**, as did Schilling and Panero (2002); McVaugh (1984), however, treated this as a questionable synonym of his var. **websteri**, and in time it might also fall within the fabric of **H. cordata**, but available collections, what with their small, few-flowered, heads, nearly glabrous leaves and restricted geography, suggest that it is worthy of formal recognition.

Schilling and Panero (2002) recognized *H. brandegei* as a distinct species, but Strother (1999) treated it as a synonym of **H. cordata**; after examination of a photograph of an isotype (MO) of the taxon concerned, I agree with Strother's appraisal.

As aptly noted by McVaugh, the entire complex is in need of more detailed field and experimental study.

HYMENOSTEPHIUM GRACILLIMUM (Brandeggee) Schill. & Panero, Bot. J. Linn. Soc. 40: 65. 2002.

Viguiera gracillima Brandeggee

Oax and Cps, shore line habitats, 5-100 m; Sep-Nov. **Map 2**

Annual, tap- rooted, herbs, 20-80 cm high. **Leaves** opposite below, alternate above, 2-8 cm long, 1-4 cm wide; petioles 1-3 cm long; blades variously ovate, the margins serrate, hispid-strigose above and below. **Capitulescence** an axillary arrangement of 1-4 heads on slender ultimate peduncles, 2-4 cm long. **Heads** 4-5 mm high; involucre 2-seriate, the bracts ca 9, lanceolate. **Ray florets** 5, neuter; ligules yellow, 2-5 mm long. **Disk florets yellow**, ca 20 per head. **Achenes** ca 3 mm long, appressed-pilose, the pappus awns 2-4 mm long, the intervening scales ca 1.5 mm long.

The species is seemingly confined to the Pacific shore lines in southeastern Oax, barely extending into closely adjacent Cps (Arriago, *Mell 2185*, LL), this not accounted for by Strother (1999).

HYMENOSTEPHIUM HINTONII (H. Rob.) Schill. & Panero, Bot. J. Linn. Soc. 140: 74. 2002.

Viguiera hintonii H. Rob.

Mic and Gue, oak woodlands, 600-1000 m; Feb-Jul. **Map 3**

Suffruticose perennial, reclining herbs or shrubs, 1-2 m high. **Leaves** 3-8 cm long, 1-3 cm wide; petioles 0.3-1.5 cm long; blades ovate-lanceolate, glabrous above and below, or nearly so, 3-nervate from the base, the margins remotely serrate. **Capitulescence** a terminal, lax, cymose panicle of 2-10 heads, the ultimate peduncles 1-10 mm long. **Involucres** 4-5 mm high, 2-3 mm wide; bracts ovate-lanceolate, imbricate, minutely pubescent. **Receptacle** convex; pales, 3.0-3.5 mm long, ca 1.5 mm wide. **Ray florets** 5, neuter; ligules yellow, 3-6 mm long, 2-3 mm wide. **Disc florets** 5-10, yellow; corolla ca 3.0 mm long; throat ca 2 mm long; lobes ca 0.5 mm long. **Achenes** ca 2.5 mm long, 0.8-1.0 wide, appressed-pubescent; pappus absent.

This taxon is distinguished from **H. cordata** by a combination of characters (glabrous, remotely serrate, leaves; relatively small heads; epappose achenes). Robinson (1977) provided a photograph of the holotype.

McVaugh (1984) placed **H. hintonii** in synonymy (albeit questionably) within his concept of *H. cordata* var. *websteri*. Indeed, **H. hintonii** appears to be closest to **H. websteri**, sharing the epappose pubescent achenes of the latter, but having smaller heads and the habit of **H. cordata**.

HYMENOSTEPHIUM SUPERAXILLARE Blake, Proc. Biol. Soc. Washington 37: 57. 1924.

Viguiera superaxillaris (Blake) B.L. Turner

Viguiera vorobikae B.L. Turner

s Chi and n Sin, pine-oak forests, 600-2000 m; Oct-Nov. **Map 1**

Shrub 1-3 m high. **Leaves** ovate, 7-12 cm long, 3-5 cm wide; petioles 1-2 cm long; blades scabrous-pubescent above and below, the margins crenulo-dentate. **Capitulescence** of 2-3 terminal heads, the ultimate peduncles scabrous-pubescent, 3-7 cm long. **Involucres** hemispheric, 3-4 seriate, 5-6 mm high, 8-10 mm across; bracts ovate-lanceolate, subequal, the outer series somewhat foliaceous and reflexed. **Ray florets** 11-13, neuter; corollas yellow, the ligules 8-12 mm long, 3-5 mm wide. **Disc florets** numerous, yellow, ca 4 mm long; tube ca 1 mm long, the limb ca 3 mm long. **Anthers** brown, ca 2 mm long, the filaments glabrous. **Achenes** black, epappose, 2.5-2.8 mm long, ca 1 mm wide.

As noted by Blake in his original description, "This species has the largest heads of any known **Hymenostephium**, and is further distinguished by its phyllaries, which are broader than in any other species and do not have the attenuate or very narrowly acuminate tips found in practically all the others." He aptly notes that it appears nearest the epappose forms of **H. cordatum**. My description of *Viguiera vorobikae* was based upon specimens clearly referable to **Hymenostephium superaxillaris**, the error corrected soon after its needless description (Turner 1990).

HYMENOSTEPHIUM TENUIS (A. Gray) Schill. & Panero, Bot. J. Linn. Soc. 140: 74. 2002.

Viguiera tenuis A. Gray

Viguiera tenuis var. *alba* Rose

Sin, Nay, Jal, Col, Mic, Mex, Mor, Gue, Oax, Cps and Guatemala southwards, tropical deciduous and pine-oak forests, 400-2000 m; Oct-Dec. **Map 4**

Erect, tap-rooted, annuals to 80 cm high. **Leaves** ovate to ovate-lanceolate, mostly 3-6 cm long, 0.8-2.5 cm wide, sessile, 3-nervate from the base, the margins entire to remotely serrate, sparsely

pubescent above and below. **Heads** radiate or rarely not (in Cps), mostly few and terminal on slender peduncles 5-20 cm long; involucre campanulate, 2-3 seriate, 6-8 mm high, 4-10 mm wide, the bracts linear-lanceolate, mostly subequal. **Ray florets** 5-21 (rarely absent), the ligules 4-8 mm long, yellow, rarely white. **Disk florets** 20-80; corollas yellow, 4-5 mm long; tubes ca 1 mm long. **Achenes** obovate, black, mostly 2-3 mm long, appressed-pubescent, the pappus of 2 awns 3-6 mm long, the intervening scales 0.8-1.6 mm long. **Chromosome number**, $n = 12$ pairs.

A rather commonly encountered, variable, species but easily recognized by its erect, annual, habit, long slender peduncles and sessile leaves.

Populational systems from along the Pacific slopes of Chiapas, so far as known, all possess rayless heads, as noted by Strother (1999), and generally somewhat longer ultimate peduncles.

HYMENOSTEPHIUM UNISERATUM Schill. & Panero, Bot. J. Linn. Soc. 140: 74. 2002.

Haplocalymma microcephalum (Greenm.) Blake

Viguiera microcephala Greenm.,

non *Hymenostephium microcephalum* (Less.) Blake

Mic, Mex, Mor, Pue and Gue, tropical deciduous forests, 300-1600 m; Oct-Nov. **Map 5**

Erect annual herbs to 1 m high. **Leaves** mostly alternate throughout, the lower-most opposite, 6-9 cm long, 3-5 cm wide; petioles 1-4 cm long; blades broadly ovate to deltoid, 3-nervate from the base, the margins coarsely dentate to nearly entire. **Capitulescence** a terminal or axillary, few-headed cyme of 1-12 heads, the ultimate peduncles mostly 0.2-1.0 cm long. **Heads** 4-5 mm high; involucre 1-2 seriate, the outer series of bracts 5, ovate-apiculate, in a single series; receptacle convex. **Ray florets** 5, neuter, the ligules yellow, 3-5 mm long. **Disk florets** yellow, 10-20 per head. **Achenes** ca 2 mm long, pubescent, the pappus awns ca 3 mm long, readily deciduous, the intervening scales ca 1 mm long. **Chromosome number**, $n = 12$ pairs (Kiel et al. 1988).

A very distinct taxon, not readily confused with another. As noted by Schilling and Panero (2002), "The epithet refers to the single row of involucral bracts, which is the distinguishing feature of the species." According to Blake, however, in his original description of *H. woronowii*, he refers to its principal generic character [within *Haplocalymma*] "as an involucre composed of only 5 phyllaries in a single series."

HYMENOSTEPHIUM WEBSTERI (B.L. Turner) Schill. & Panero, Bot. J. Lin. Soc. 140: 74. 2002.

Hymenostephium kingii (McVaugh) Schill. & Panero

Viguiera cordata var. *websteri* (B.L. Turner) McVaugh

Viguiera kingii McVaugh

Viguiera websteri B.L. Turner

Nay and Jal, 1000-1800 m; Aug-Mar. **Map 3**

Perennial, stiffly erect, herbs 30-80 cm high, arising from a corm-like tap root. **Leaves** mostly 4-7 cm long, 2-4 cm wide; petioles 2-6 mm long; blades broadly lanceolate, the margins serrate, sparsely pubescent above and below. **Capitulescence** a terminal array of 3-10 divergent heads, the ultimate peduncles 2-5 cm long. **Involucre** 5-12 mm high, 4-12 mm wide. **Ray florets** 8, neuter; ligules yellow, 8-10 mm long. **Disk florets** 20-50 per head; corollas yellow ca 5 mm long; throat ca 3-4 mm long. **Achenes** black, 3-4 mm long, 1.0-1.3 mm wide, appressed pubescent, somewhat obtusely 4-angled; pappus absent. **Chromosome number**, $n = 17$ pairs (Turner, Powell and King 1962).

This is a rarely encountered, very distinct species, known to me by only five collections: the Type (Nay, 25 km S of Tepec), and *McVaugh 16599* (Type of *H. kingii*, MICH !), *Panero 2229* (LL); Carrillo-Reyes 2922 (TEX), all collected in the vicinity of Tepec, Nayarit; *Breedlove 60633* (CAS) [Jal, Mpio. Atenguillo, 15 km NW of Los Volcanes, 1890 m, Nov 1983].

The taxon is readily distinguished from its closest cohort, **H. cordata**, by its smaller habit and corm-like roots, larger involucre, and diploid chromosome number (vs polyploid).

McVaugh (1984) inexplicably treated *H. kingii* as distinct from **H. websteri**; at least I can find no characters that might distinguish between them; he also provided an excellent sketch of **H. websteri** (based upon the type specimen of *H. kingii*). Schilling and Panero (2002; 2011), however, recognized both *H. kingii* and **H. websteri** as distinct species.

HYMENOSTEPHIUM WORONOWII (Blake) Schill. & Panero, Bot. J. Linn. Soc. 140: 74. 2002.

Haplocalymma woronowii Blake

Viguiera woronowii (Blake) H. Rob.

Known only from Mic, Uruapan and vicinity, pine-oak forests, 1600-1700 m; Aug-Jan. **Map 6**

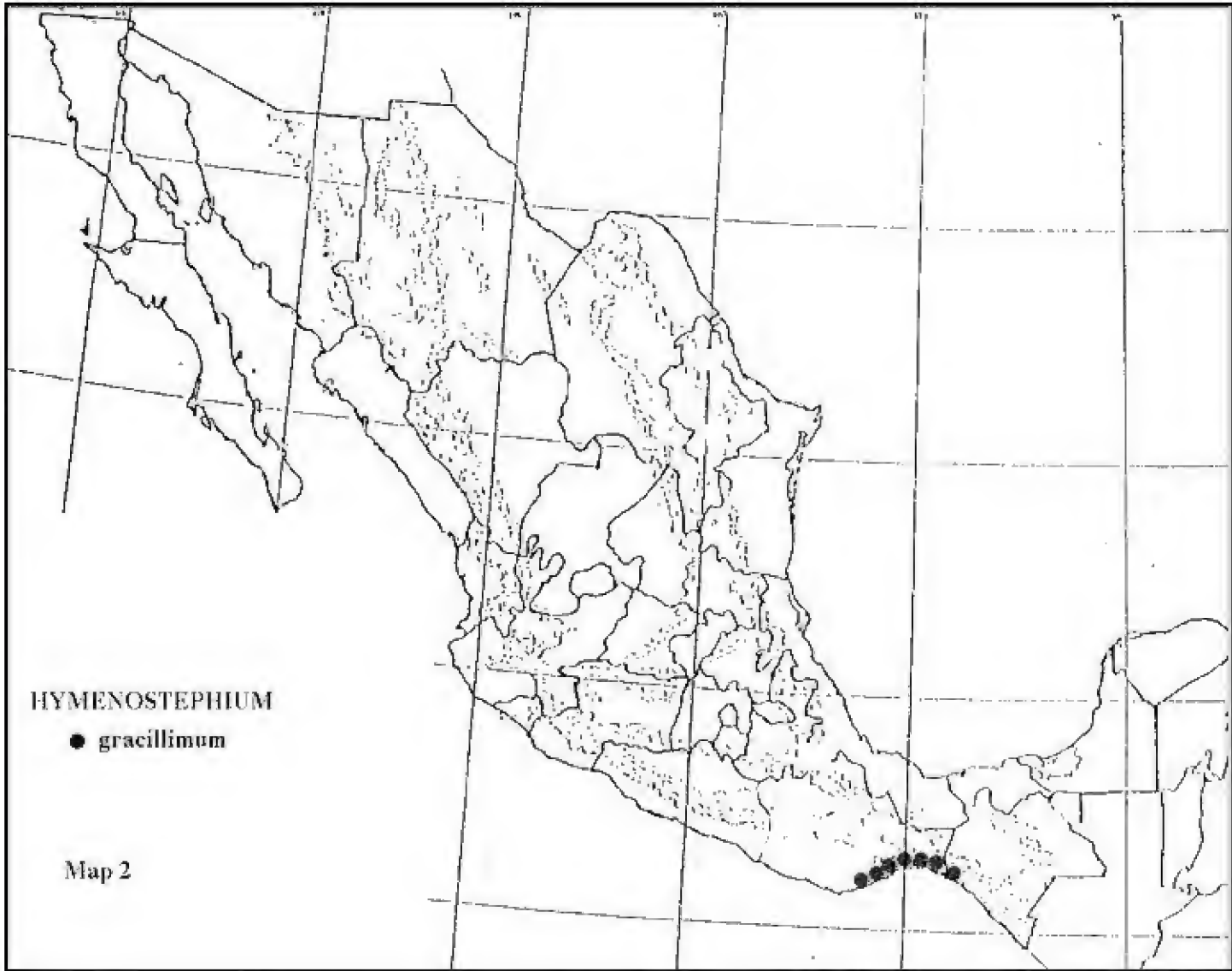
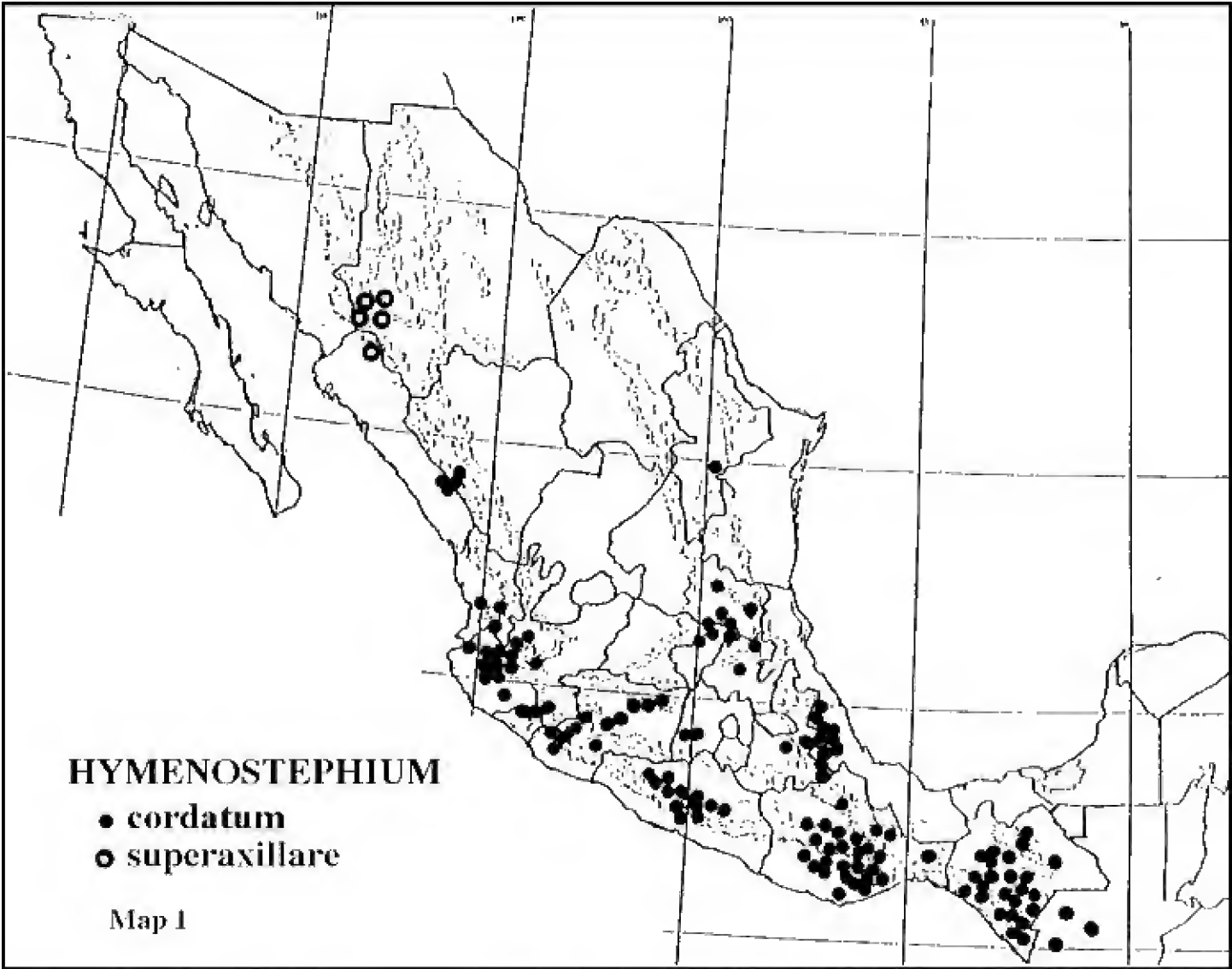
Annual, slender-stemmed, herbs to 50 cm high. **Leaves** opposite below, alternate in the inflorescence, 2-3 cm long, 1.0-1.5 cm wide; blades lanceolate, hirsute-pilose on both surfaces, the margins serrate; petioles 1-2 mm long. **Inflorescence** a terminal cymose panicle of 4-10 heads, 4-8 cm wide, 4-5 cm high, the ultimate peduncles mostly 2-6 cm long. **Heads** relatively small, 5-6 mm across (the rays excluded), 2-4 mm high. **Involucre**s reportedly uniseriate (on the type) to biseriate, the phyllaries lanceolate, ca 1 mm wide. **Receptacle** conical, ca 1.5 mm high, 1 mm wide; pales broadly lanceolate, ca 2.5 mm long, their apices acute. **Ray florets** 5, neuter, reportedly yellow; ligules 5-7 mm long, 4-5 mm wide. **Disk florets** ca 20 per head; corollas ca 2.3 mm long; throat ca 1.5 mm long. **Achenes** glabrous, epappose, ca 1 mm long.

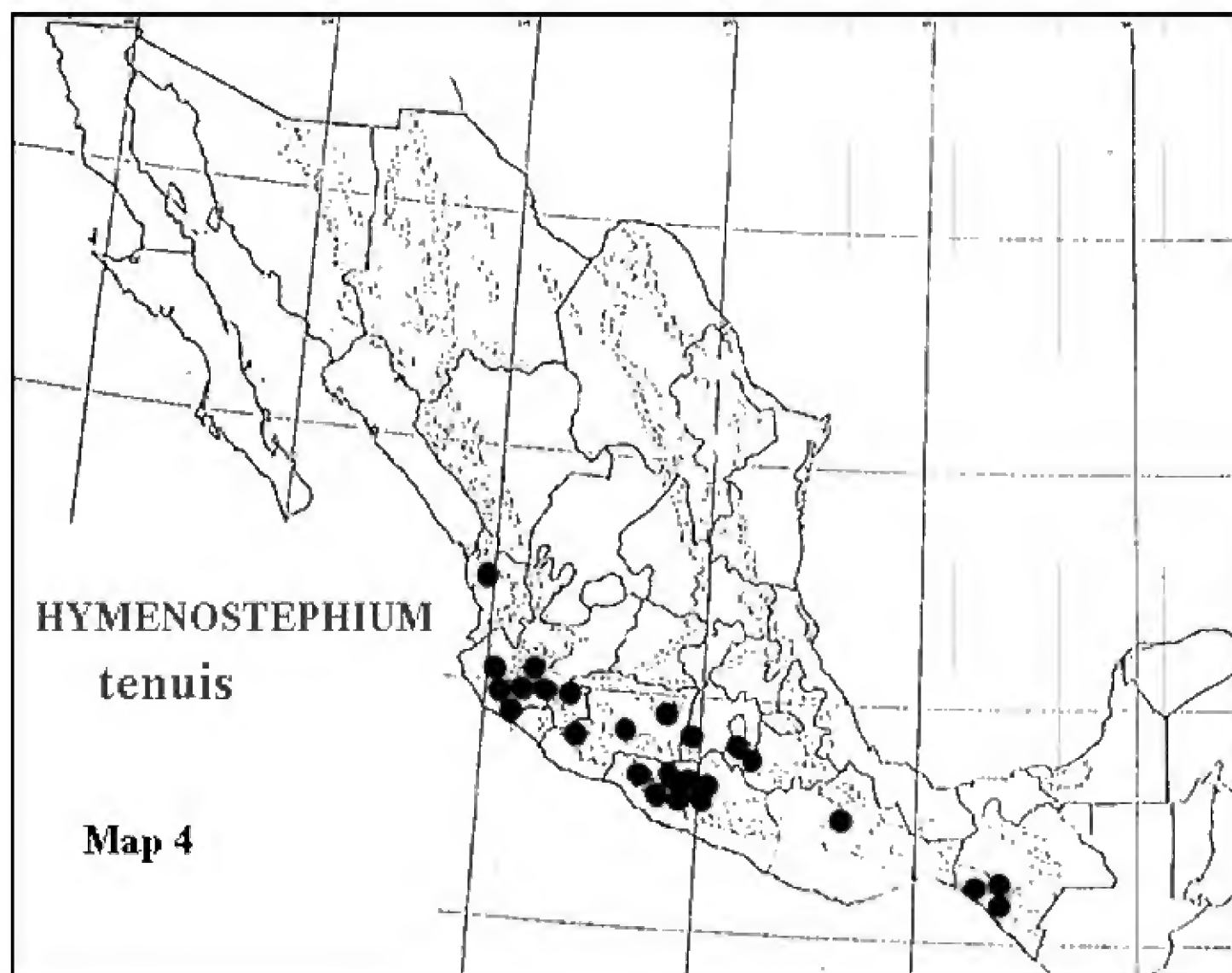
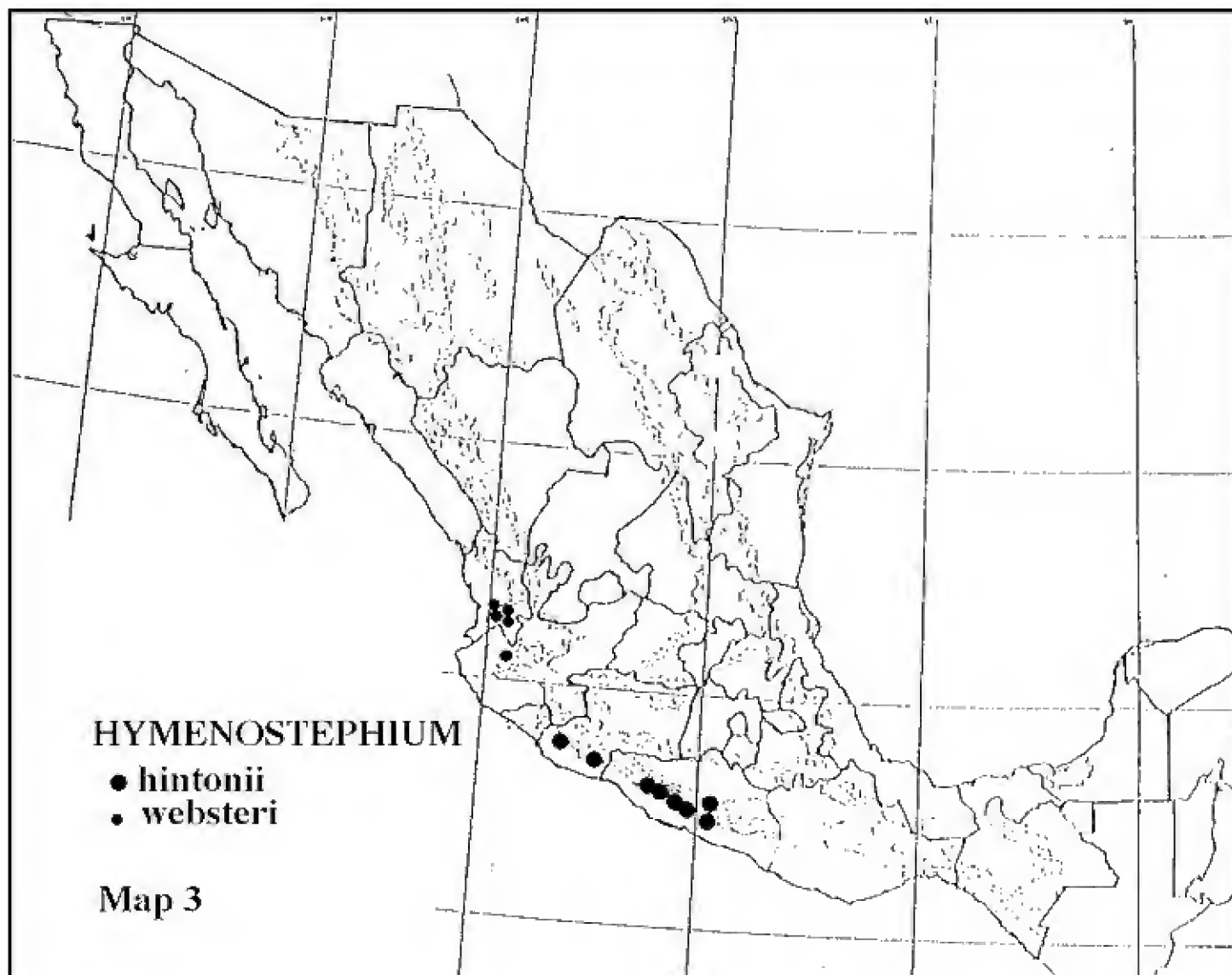
In the transfer of *Haplocalymma woronowii* into **Viguiera**, Robinson (1977) called attention to the anomalous conical receptacle of this taxon, which is its most distinguishing feature. Additionally, it has very small (ca 1 mm long) epappose, glabrous, achenes and smaller disc florets, with shorter throats, than other members of the **Hymenostephium** complex.

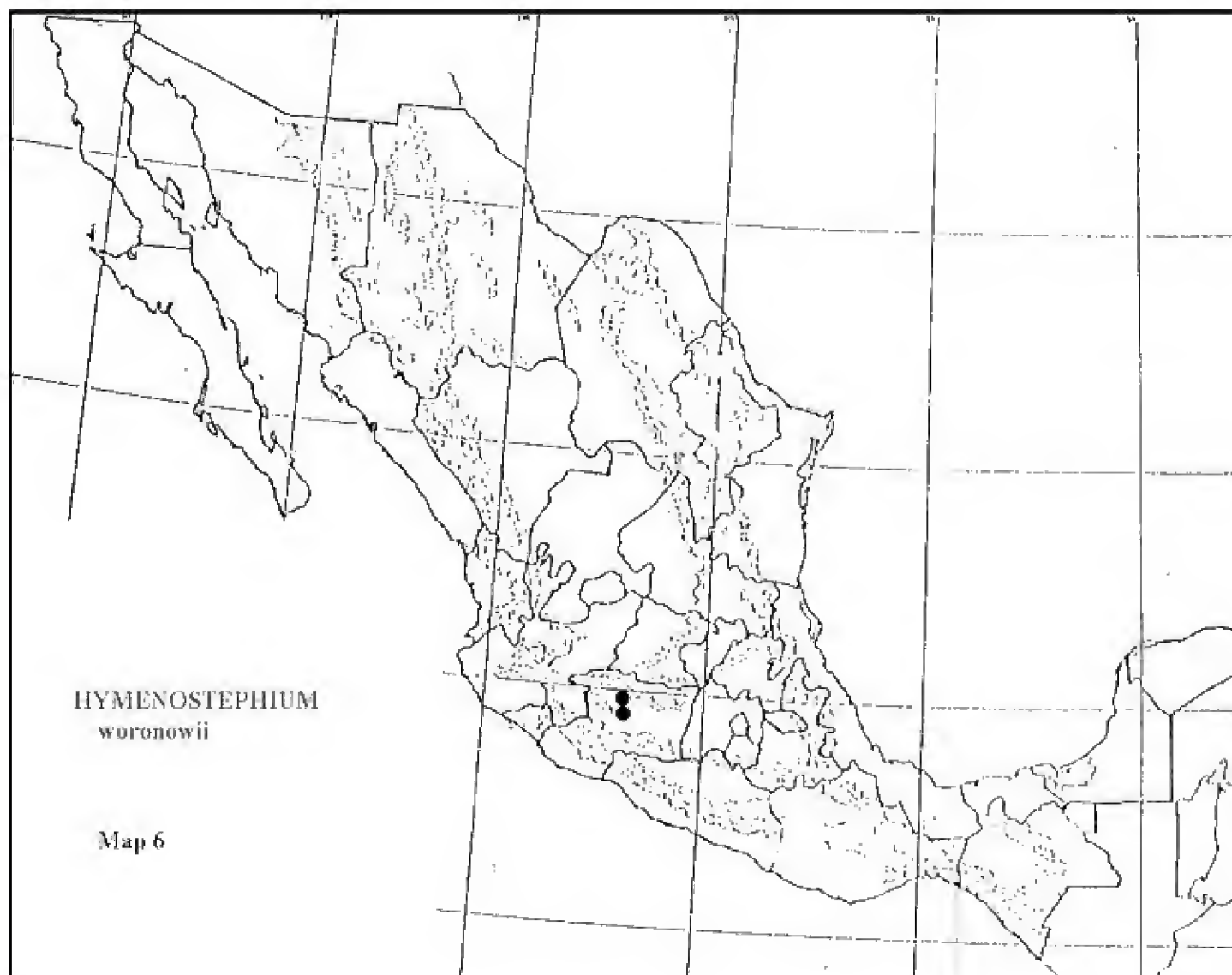
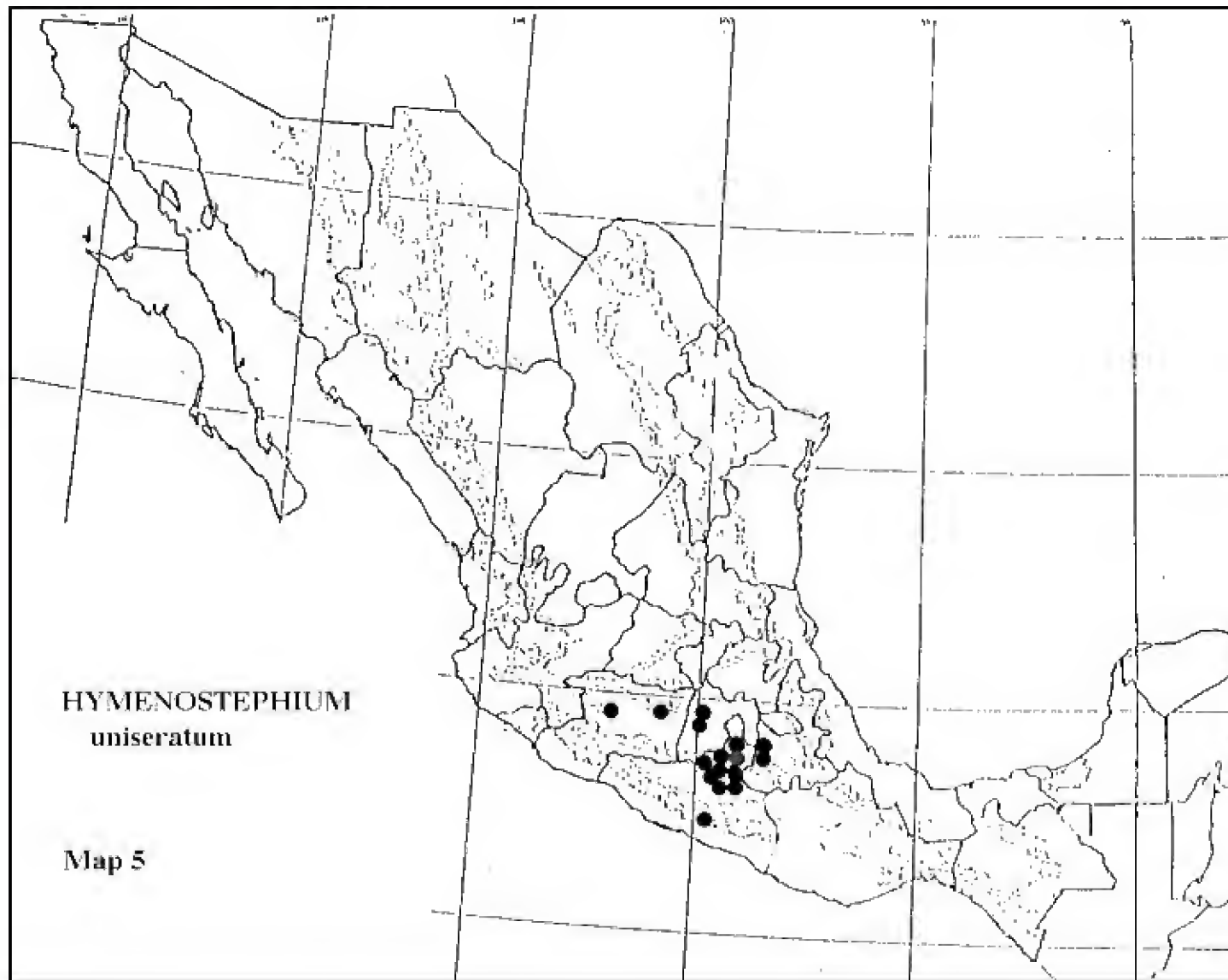
ACKNOWLEDGEMENTS

My long time companion, Jana Kos, edited the paper and provided helpful input. The dot maps are based upon specimens at LL-TEX, and those called to the fore by various published works.

LITERATURE CITED [cf. references, above]







Chemosystematics of *Juniperus*: Effects of leaf drying on the essential oil composition of *Juniperus pinchotii*

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ABSTRACT

A bulk collection of terminal branchlets was made from *J. pinchotii* and subjected to drying at 42°C (24 hrs), then stored for up to 24 mos. at 22°C (room temperature, RT). The oils were distilled and analyzed from fresh, 0.5, 1, 2, 4, 8, 16, and 24 mos. storage at RT. The oil yields showed a slight decline initially, but remained fairly constant. Camphor, camphene hydrate and citronellal declined (mg/g dry foliage) in fresh vs. 0.5 mo. samples. Borneol increased during storage (on a mg/g basis). This may be due to the loss of acetate by bornyl acetate and/ or oxidation of terpenes to produce borneol. Overall, most of the changes occurred between the fresh and 0.5 mo. samples. It appears one can use the oils from dried leaves of *Juniperus pinchotii* for geographical studies, but mixing fresh and dried leaf samples may present a problem for this taxon. Published on-line: www.phytologia.org *Phytologia* 95(1): 10-17 (Feb. 1, 2013).

KEY WORDS: *Juniperus pinchotii*, oils from dried leaves, chemosystematics, terpene decomposition.

With the importation of fresh plant materials into the USA (and other countries) becoming increasingly difficult due to plant quarantine laws, it is often necessary to utilize specimens that have frozen to kill insects and then air dried. However, the composition of the oils from air dried leaves may change during drying.

Achak et al. (2008, 2009) compared the leaf essential oils from fresh and air dried (22° C, 16 days) leaves for *J. thurifera* L., *J. phoenicea* L. and *J. oxycedrus* L. The first two species are in section *Sabina* and have scale-leaves, whereas *J. oxycedrus* is in section *Juniperus* with awl-like leaves (Adams, 2011). They reported small to moderate changes in several components, however, no statistical data were published.

Adams (2010) reported that the composition of *J. virginiana* leaf oils from specimens stored at room temperature (22° C) for up to 8 mos. were very stable. However, (Adams, 2011) later reported considerable differences in oil from *J. virginiana* leaves stored for 16 mos. Adams (2010) also examined the leaf oils of *J. pinchotii* from fresh and air dried for 2 weeks and found that oil yield declined from 1.45% to 1.10% (w/w, oven dry wt. basis). In addition, borneol increased and citronellal decreased (highly significantly) from fresh to 2 wk. at room temperature (22° C). Camphor significantly increased from fresh to dried leaves. The concentration of 4 other components changed from fresh to dried leaf oils (Adams, 2010).

The purpose of this study is to report on changes in composition of leaf oils from *J. pinchotii* leaves stored for long term (up to 24 mo.) at room temperature (22° C).

MATERIALS AND METHODS

Plant material - *J. pinchotii*, Adams 12289, 10 mi. s of Post on RR 669, Garza Co., TX. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g) and air dried (100 g) leaves were co-steam distilled with 20 mg of undecane (internal standard) for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principal component analysis (PCA) follows Veldman (1967).

RESULTS AND DISCUSSION

Comparisons of the leaf components (on a mg/g foliage oven dry weight basis) from the leaves of *J. pinchotii* from fresh vs. air dried (42° C, 24 hr) then stored at room temperature (22° C) for 0.5 to 24 mo. are shown in Table 1. Of major interest are the changes in yield which varies little over the 24 mo. (Fig. 1). The yield appears to decrease from fresh to 0.5 mo., then shows an unusual decline at 2 mo. (Fig. 1). However, this may be due to sub-sampling. The leaf branchlets were pressed and stored in newspapers at RT. When the samples were distilled, entire branch-let, consisting of woody stems (up to ~ 3 mm cx.) with attached leaves were distilled. It may be that in the 2 mo. sample, a greater proportion of the woody stems were included. Because the oil is found chiefly in the leaves, not the wood, this could have led to a 'decline' in yield.

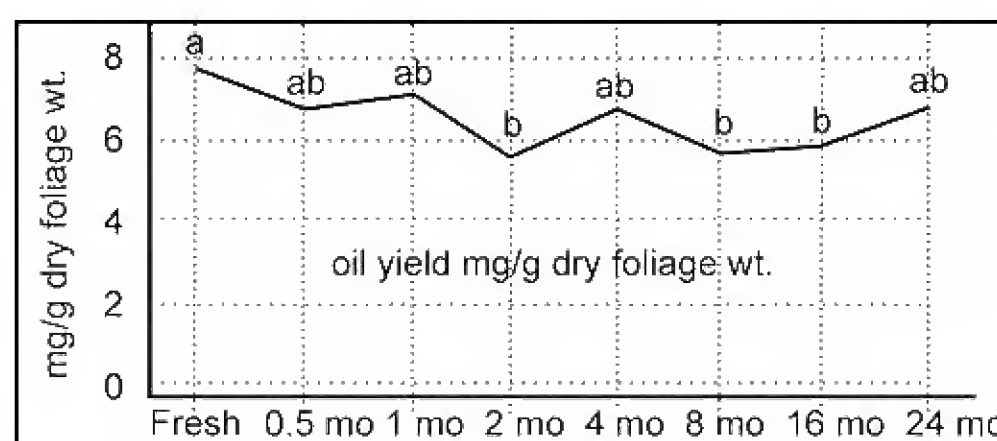


Figure 1. Variation in oil yield (mg/g) over a 24 mo. period. Any sample sharing a common letter is not statistically different.

The major volatile leaf oil components have highly significant differences (Table 1), except terpinolene (significant) and two components that were not significant (α - and γ -terpinene). Several compounds declined between fresh and 0.5 mo. drying (Fig. 2, camphor, camphene hydrate, citronellal). The decrease in camphor (Fig. 2) is significant, but subsequent changes are not significant. This pattern is also seen in camphene hydrate and citronellal (Fig. 2, Table 1). The spike in camphene hydrate at 4 mo. is unexplained.

Variation in monoterpene hydrocarbon components tended to have similar patterns (Fig. 3). Limonene was significantly larger in samples from 1 mo. and 4 mo. and lower in 8 and 16 mos. (Fig. 3). γ -terpinene displayed no significant differences. Myrcene was stable with a decline after 4 mos. (Fig. 3). α -thujene increased from the fresh to 0.5 mo. samples and then displayed mostly steady concentrations (Fig. 3.).

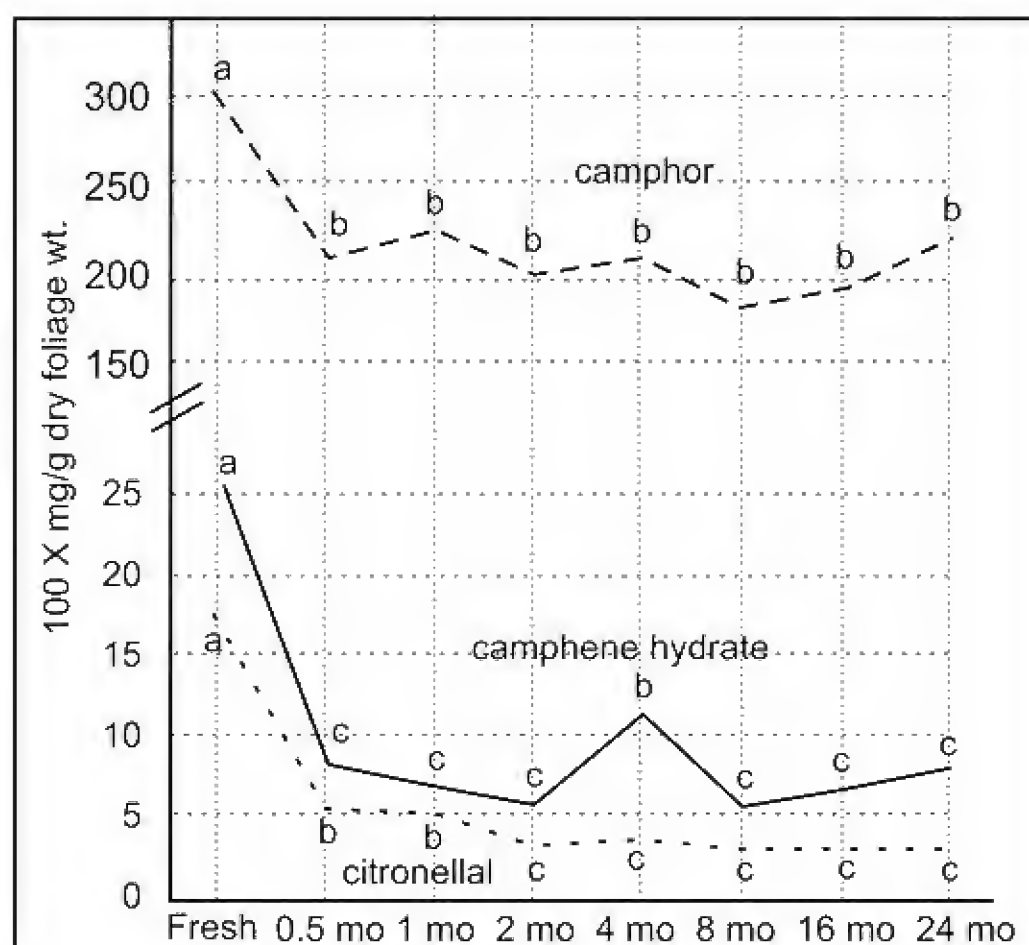


Figure 2. Variation in camphor, camphene hydrate and citronellal. Data points with different letters are significantly different. Data points with the same letter are not significantly different ($P = 0.05$).

One of the few compounds that increased was borneol (Fig. 4). This may, in part, be due to deacetylation of bornyl acetate that declined (Fig. 4). *cis*-sabinene hydrate declined initially, and then remained relatively stable (Fig. 4).

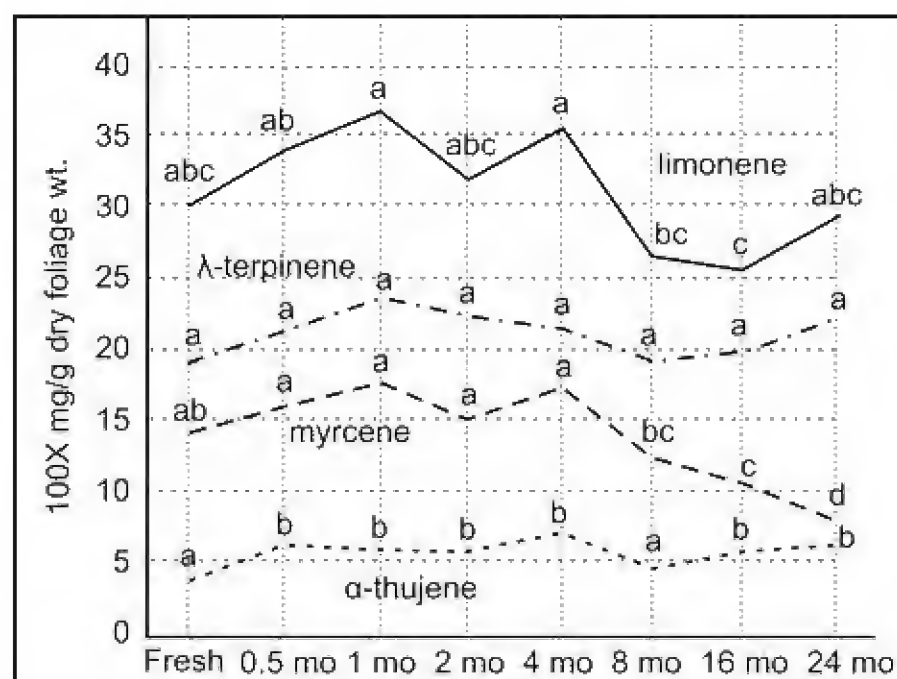


Figure 3. Variation among monoterpene hydrocarbons. Significance is as defined in Figure 2.

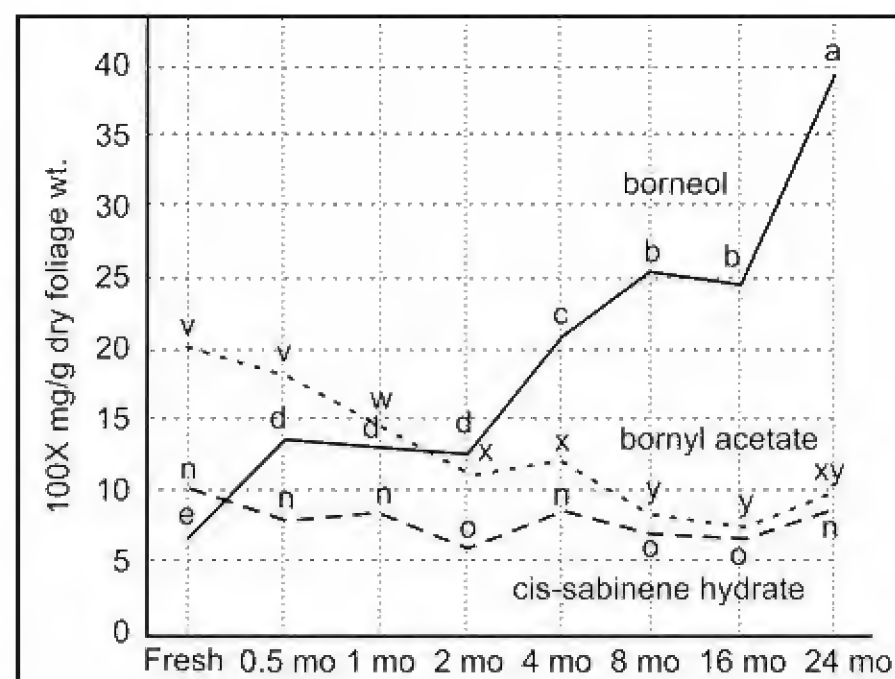


Figure 4. Variation in borneol, bornyl acetate and *cis*-sabinene hydrate. Note the inverse relationship between borneol and bornyl acetate.

Principal components analysis (PCA) of the 19 terpenoids and oil yields gave 3 eigenroots accounting for 31.1, 23.8 and 19.0% of the variance among these components. Plotting these three components reveals clustering by chemical classes except for oil yield, borneol, and bornyl acetate (Fig. 5). If borneol is increasing at the expense of bornyl acetate, that could explain their negative correlation (-0.69). Generally, the monoterpene hydrocarbons (C10-HC, Fig. 5) are in a group. Other groups are the oxygenated terpenes (C10-oxy, Fig. 5) and most of the sesquiterpene alcohols (C15-OH, Fig. 5).

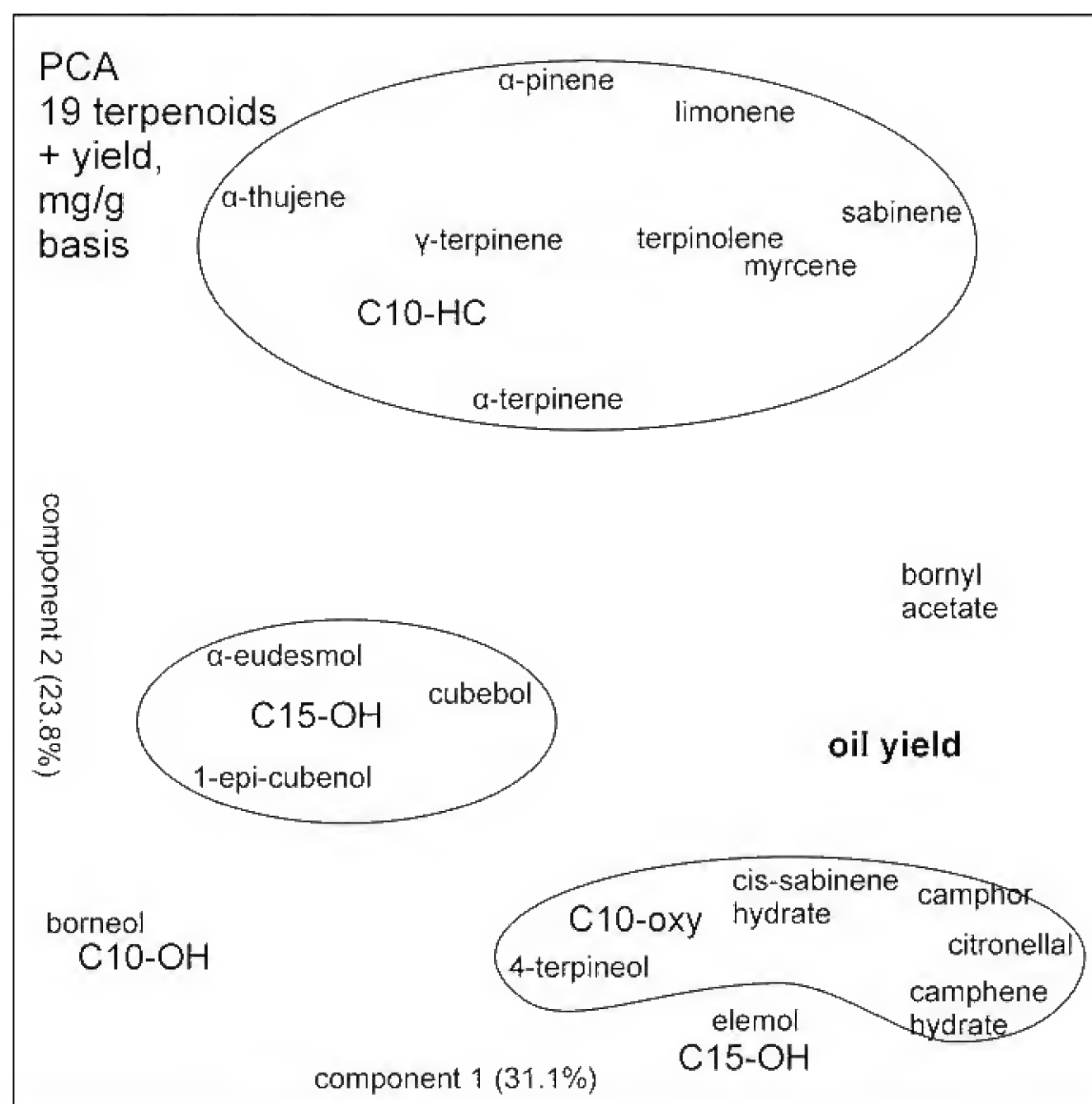


Figure 5. PCA of 19 terpenoids and oil yield (on a mg/g basis).

Juniperus pinchotii is in the serrate-leaf margined *Juniperus* group and has oil glands that rupture with white exudate on the leaves (Fig. 6). Fresh leaves were washed with diethyl ether and the wash compared with the oils from fresh leaves (Table 2). The major components of the ether wash were diterpenoids: sclareol, diterpene 2268, methyl abietate isomer, and an unknown diterpene acid (Table 2), none of these were found in the leaf oil. Camphor (40% in leaf oil) was 4.7% and bornyl acetate (2.7% in leaf oil) was 4.1%. The more volatile monoterpenes were absent or very small in the leaf wash, as one would expect from long-term exposure to ambient conditions. Several compounds absent in the leaf oil were found in the leaf wash: karahanaenone, p-cymen-8-ol, trans-sabinene hydrate acetate, trans-calamenene, sclareol, diterpene 2268, sempervirol diterpene acid 2408 and methyl abietate isomer (Table 2). It is not if the leaf glands ruptured, then sealed or continue to exude or 'bleed' components. It is interesting that not all of the leaves (Fig. 6) have ruptured glands. Gland rupturing may be a natural defense mechanism or a wound/ pathogen response.



Figure 6. Gland exudate in *J. pinchotii*.

The amount of changes between the oils from fresh and dried leaves of *J. pinchotii* (this study) is much greater than found in *J. virginiana* (Adams, 2010). To investigate the potential systematic use for the *J. pinchotii* oils, PCO was performed on the oils from the 8 storage tests and compared with oils from *J. ashei*. PCO ordination reveals that the *J. pinchotii* oils do cluster, but the fresh oil is somewhat different (Fig. 6). In addition, the 2 mo. oils show some differences (Fig. 6).

It appears that both the fresh and dry leaf oils of *J. pinchotii* could be used for chemosystematic studies involving *J. ashei* and likely other species. However, it also appears that for studies of geographic variation in *J. pinchotii*, either fresh or dried leaves could be used, but not both, as the differences may be large enough to mask geographical trends.

Clearly, additional studies would be useful to ascertain the changes found in the oils from fresh vs. air dried leaves stored for only 0.5 mo. These results are surprising, considering the mild drying and storage conditions used in this study.

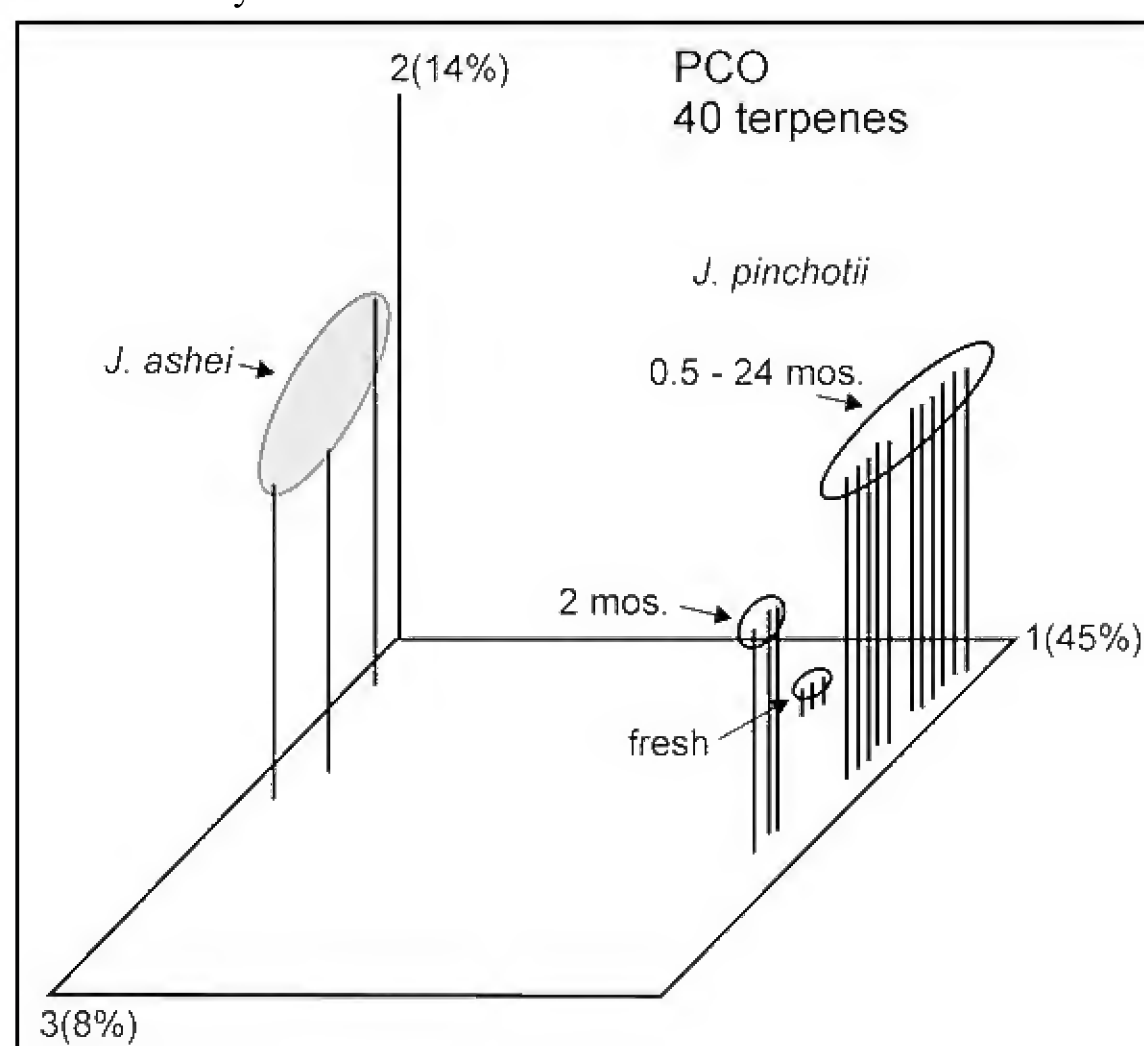


Figure 6. PCO based on 40 terpenoids for *J. ashei* and *J. pinchotii* stored for up to 24 mo.

ACKNOWLEDGEMENTS

Thanks to Art Tucker and Billie Turner for reviews. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Table 1 Comparison of leaf oils (100 X mg/g basis) for major components obtained from fresh leaves of *J. pinchotii* vs. leaves dried and stored at 21° C for 0.5, 1, 2, 4, 8, 16 and 24 mos. ODW = oven dry wt. of extracted foliage. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

KI	Compound	Fresh	0.5 mo	1 mo	2 mo	4 mo	8 mo	16 mo	24 mo	F sig
	yield mg/g ODW	7.5	6.7	7.0	5.6	6.7	5.6	5.8	6.7	*
924	α -thujene 100X (mg/g)	3.6	6.0	5.8	5.7	6.7	4.5	5.5	5.9	**
932	α -pinene	5.6	8.1	8.1	7.0	8.5	6.4	6.0	6.2	**
969	sabinene	113	122	121	103	126	82.2	73.5	80.4	**
988	myrcene	14.2	16.6	17.6	15.0	16.8	11.9	10.8	7.6	**
1014	α -terpinene	11.5	12.7	14.2	12.9	13.1	11.4	11.6	12.8	ns
1024	limonene	30.0	33.6	36.7	31.9	35.2	26.5	25.2	29.5	**
1054	γ -terpinene	18.9	20.9	23.2	22.2	21.5	19.0	19.6	22.1	ns
1065	cis-sabinene hydrate	9.8	7.9	8.2	5.3	8.1	6.8	6.8	8.8	**
1086	terpinolene	7.5	8.2	9.2	8.1	8.3	6.8	6.9	7.9	*
1141	camphor	300	209	223	199	207	179	191	220	**
1145	camphene hydrate	24.5	8.0	6.7	5.8	10.9	5.4	6.3	7.6	**
1148	citronellal	17.0	5.4	5.2	3.2	3.4	3.0	2.8	2.8	**
1165	borneol	6.5	13.4	12.7	11.8	21.1	26.2	23.9	39.1	**
1174	terpinen-4-ol	54.0	44.2	51.9	42.0	53.3	45.5	52.9	61.6	**
1284	bornyl acetate	20.3	18.3	14.7	11.0	11.9	8.1	7.5	9.6	**
1514	cubebol	5.4	7.4	7.2	3.4	6.2	6.3	5.8	8.2	**
1548	elemol	10.6	8.0	9.3	4.8	7.2	7.1	8.3	9.5	**
1627	1-epi-cubenol	3.8	5.6	6.8	4.1	5.6	6.3	7.0	7.8	**
1652	α -eudesmol + α -cadinol	4.1	6.0	8.5	3.6	6.2	7.0	7.9	6.5	**

KI = Kovats Index (linear) on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2 Comparison of components (percent total oil) from fresh leaves of *J. pinchotii* vs. ether wash of exudate.

KI	Compound	Fresh	ether wash
921	tricyclene	0.3	t
924	α -thujene	0.5	0.3
932	α -pinene	0.7	t
946	camphene	0.4	t
969	sabinene	15.1	0.9
974	β -pinene	0.1	-
988	myrcene	1.9	-
1002	α -phellandrene	t	-
1014	α -terpinene	1.5	t
1020	p-cymene	0.1	0.2
1024	limonene	4.0	0.2
1054	γ -terpinene	2.5	0.2
1065	cis-sabinene hydrate	1.3	0.4
1086	terpinolene	1.0	t
1098	trans-sabinene hydrate	0.2	0.5
1118	cis-p-menth-2-en-1-ol	0.4	-
1141	camphor	40.0	4.7
1145	camphene hydrate	3.3	0.2
1148	citronellal	2.3	-
1154	karahanaenone	-	0.3
1165	borneol	0.9	0.3
1174	terpinen-4-ol	7.2	0.2
1179	p-cymen-8-ol	-	0.2
1186	α -terpineol	0.4	0.3
1195	cis-piperitol	t	-
1207	trans-piperitol	0.1	-
1219	coahuilensol, me-ether	0.1	-
1223	citronellol	4.6	0.4
1253	trans-sabinene hydrate acetate	-	0.2
1274	pregeijerene B	t	-
1284	bornyl acetate	2.7	4.1
1451	trans-muurola-3,5-diene	0.2	0.3
1475	trans-cadina-1(6),4-diene	0.1	0.1
1493	trans-muurola-4,5-diene	0.4	0.4
1493	epi-cubebol	0.2	0.5
1514	cubebol	0.7	2.9
1521	trans-calamenene	-	0.7
1522	δ -cadinene	0.3	-
1548	elemol	1.4	0.8
1627	\square -epi-cubenol	0.5	0.6
1630	γ -eudesmol	0.1	t
1649	β -eudesmol	0.2	0.3
1652	α -eudesmol + α -cadinol	0.6	0.3
1792	8- α -acetoxyelemol	0.2	0.6
1987	manoyl oxide	0.1	2.2
2055	abietatriene	t	1.2
2087	abietadiene	0.2	0.8
2222	sclareol	-	13.0
2268	diterpene alcohol or aldehyde	-	2.1
2282	sempervirol	-	1.6
2298	4-epi-abietal	0.2	3.1
2312	abieta-7,13-dien-3-one + abietal	0.3	7.2
2408	diterpene acid	-	4.2
2444	methyl abietate isomer	-	5.4

Table 3. Comparison of components (percent total oil) obtained from fresh leaves of *J. pinchotii* vs. leaves dried and stored at 21° C for 0.5, 1, 2, 4, 8, 16 and 24 mos. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested.

KI	Compound	Fresh	0.5 mo	1 mo	2 mo	4 mo	8 mo	16 mo	24 mo	F sig
	percent yield (% ODW)	0.75	0.67	0.70	0.56	0.67	0.56	0.58	0.67	*
921	tricyclene	0.25	0.48	0.35	0.48	0.50	0.64	0.35	0.36	nt
924	α -thujene	0.48	0.89	0.83	1.02	1.00	0.98	0.94	0.88	**
932	α -pinene	0.74	1.21	1.15	1.25	1.27	1.15	1.03	0.93	**
946	camphene	0.40	0.57	0.48	0.61	0.61	0.53	0.47	0.48	nt
969	sabinene	15.12	18.25	17.32	18.43	18.79	14.67	12.67	12.00	**
974	β -pinene	0.10	0.11	0.10	0.10	0.12	0.10	0.10	0.11	nt
988	myrcene	1.89	2.48	2.52	2.68	2.50	2.13	1.87	1.13	**
1002	α -phellandrene	t	t	t	0.20	t	0.10	t	t	nt
1014	α -terpinene	1.53	1.90	2.03	2.30	1.96	2.03	2.00	1.91	*
1020	p-cymene	0.10	0.36	0.34	0.47	0.47	0.66	0.64	0.62	**
1024	limonene	4.01	5.02	5.24	5.70	5.25	4.74	4.35	4.40	**
1054	γ -terpinene	2.52	3.12	3.32	3.79	3.21	3.39	3.38	3.30	*
1065	cis-sabinene hydrate	1.30	1.18	1.17	0.94	1.21	1.22	1.18	1.31	*
1086	terpinolene	1.00	1.23	1.31	1.44	1.24	1.22	1.19	1.18	*
1098	trans-sabinene hydrate	0.20	0.33	0.40	0.10	0.10	0.20	0.20	0.44	nt
1118	cis-p-menth-2-en-1-ol	0.38	0.42	0.54	0.47	0.41	0.46	0.57	0.62	nt
1141	camphor	40.01	31.15	31.80	32.51	30.96	32.02	32.85	32.91	*
1145	camphene hydrate	3.27	1.20	0.96	1.04	1.62	0.97	1.08	1.14	**
1148	citronellal	2.27	0.80	0.74	0.54	0.51	0.53	0.49	0.42	**
1165	borneol	0.86	2.00	1.82	2.11	3.15	3.60	4.12	5.83	**
1174	terpinen-4-ol	7.20	6.60	7.41	7.50	7.96	8.12	9.12	9.20	*
1186	α -terpineol	0.43	0.40	0.43	0.37	0.41	0.43	0.47	0.51	nt
1195	cis-piperitol	t	t	t	t	t	t	t	t	nt
1207	trans-piperitol	0.12	0.21	0.20	0.10	0.20	0.12	0.22	0.33	nt
1219	coahuilensol, me-ether	0.10	t	t	t	t	t	t	t	nt
1223	citronellol	4.62	3.30	3.67	3.14	3.17	3.40	3.59	3.23	nt
1274	pregeijerene B	t	t	t	t	t	t	t	t	nt
1284	bornyl acetate	2.71	2.73	2.10	1.96	1.78	1.44	1.29	1.43	**
1298	carvacrol	t	t	t	t	t	t	t	t	nt
1374	α -copaene	t	t	t	t	t	t	t	t	nt
1451	trans-muurolo-3,5-diene	0.18	0.42	0.41	0.53	0.35	0.45	0.46	0.40	nt
1475	trans-cadina-1(6),4-diene	0.08	0.41	0.44	0.62	0.34	0.48	0.49	0.39	nt
1493	trans-muurolo-4,5-diene	0.41	1.10	1.11	1.38	1.01	1.29	1.32	1.12	**
1493	epi-cubebol	0.19	0.29	t	t	0.33	0.33	0.34	0.33	nt
1500	α -muurolene	t	t	t	0.20	0.10	0.21	0.22	0.22	nt
1514	cubebol	0.72	1.10	1.03	0.61	0.93	1.12	1.00	1.22	**
1522	δ -cadinene	0.30	1.20	1.19	1.60	1.12	1.47	1.59	1.53	**
1528	zonarene	t	0.25	0.31	0.30	0.20	0.33	0.34	0.22	nt
1548	elemol	1.41	1.20	1.33	0.85	1.08	1.27	1.43	1.42	**
1627	1-epi-cubenol	0.50	0.84	0.97	0.74	0.84	1.12	1.20	1.17	**
1630	γ -eudesmol	0.08	0.33	0.41	0.44	0.60	0.42	0.45	0.46	**
1649	β -eudesmol	0.20	0.58	0.78	0.44	0.63	0.94	1.06	0.73	**
1652	α -eudesmol + α -cadinol	0.55	0.90	1.21	0.65	0.93	1.25	1.37	0.97	**
1670	bulnesol	t	t	t	t	t	t	t	t	nt
1792	8- α -acetoxylemol	0.23	0.22	0.33	0.10	0.22	0.28	0.25	0.26	nt
1987	manoyl oxide	0.09	0.20	t	t	t	t	t	t	nt
2055	abietatriene	t	t	t	t	t	t	t	t	nt
2087	abietadiene	0.23	0.30	t	t	t	t	t	t	nt
2298	4-epi-abietal	0.21	0.40	0.49	0.11	0.37	0.48	0.49	0.59	**
2312	abieta-7,13-dien-3-one + abietal	0.33	0.58	0.88	0.27	0.59	0.77	0.78	0.92	**

A new species of *Adenophyllum* (Asteraceae: Tageteae) from northwestern Mexico

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ABSTRACT

A new taxon of *Adenophyllum* from near Yecora, Sonora, ***A. yecoranum*** B.L. Turner, **sp. nov.** is described. It belongs to the *A. porophyllum* complex, as treated by Strother (1969, 1986), where it relates to *A. cancellatum*. A photograph of the holotype is presented, and distribution maps of the complex in Mexico are provided. Published on-line: www.phytologia.org *Phytologia* 95(1): 18-22 (Feb. 1, 2013).

KEY WORDS: Asteraceae, Tageteae, *Adenophyllum*, *Dyssodia*, Mexico, Sonora

Preoccupation with the identification of Mexico Asteraceae has occasioned the present paper.

ADENOPHYLLUM YECORANUM B.L. Turner, **sp. nov.** Fig.1

Superficially resembling ***A. cancellatum***, but having smaller, yellow (vs orange) ray florets, smaller heads with fewer disc florets, and achenes w/o an outer pappus of short erose scales.

Annual, stiffly erect, herbs to 1 m high. **Leaves** alternate, glabrous, 5-9 pinnatisect, mostly 4-7 cm long, 2-3 cm wide, their apices terminated by slender flagella, 4-7 mm long. **Heads**, ca 1.5 cm high, 1 cm wide (rays excluded), borne upon bracteate peduncles 5-10 cm long. **Calyculum** of relatively few, pectinate, bracts, 3-7 mm long, the pustulate glands 0.5-1.5 mm long. **Involucral bracts** (innermost) usually ca 15, ca 1 cm long, united for ca ½ their length, bearing 3-6 glands near their apices, the upper most usually keeled. **Receptacle**, convex, alveolate-ciliate (rarely not). **Ray florets**, 8-11, pistillate, fertile; ligules yellow, 6-8 mm long, 2-3 mm wide. **Disc florets** 30-40 per head; corollas 5-6 mm long; tube ca 1.5 mm long, grading into the throat, the lobes narrowly lanceolate, ca 1.5 mm long, their apices purplish. **Achenes** ca 4 mm long, 4-sided, black, sparsely pubescent to glabrous; pappus of ca 16 squamellae dissected into 5-10 bristles, an outer series absent.

TYPE: MEXICO. SONORA: “Rio Maycoba at Mex. 16 (20.5 km west of Maycoba, 28.6 km east of Yecora), 28 22 15 N, 108 45 30 W, 1220 m, 26 Sep 1998, A.L. Reina G. 98-1732 [with T.R. Van Devender] (Holotype, TEX).

According to its collectors (pers. comm.), the holotype was collected in a grassland/oak woodland transition at the Río Maycoba.

ADDITIONAL SPECIMENS EXAMINED: MEXICO. CHIHUAHUA: Guasaremos, Rio Mayo, “oak swales and slopes,” 26 Sep 1935, *Gentry 1863* (TEX); Nabogame, 28 30 N, 108 30 W, 1800 m, Sep 1988, *Laferriere 1975* (TEX). **SONORA:** “north slopes of Mesa del Campanero, 4.8 km west of Puerto de la Cruz, 1640 m, 8 Sep 1996, *Van Devender 96-558* (TEX). **Map 1**

Strother (1969), to judge from his citations, distribution maps and annotations (at TEX), did not examine material of this novelty.

Label data on the type itself list the rays as “orange yellow,” but they appear to be yellow, not a hint of orangeness. The general area of Yécora, Mexico, and closely adjacent Chihuahua, harbor a number of rather localized endemics such as *Ageratina yecorana* B.L. Turner, *Arceuthobium yecorens* Hawksworth & Wiens, *Brickellia enigmatica* B.L. Turner, *Erigeron reinana* G.L. Nesom, *Lepechinia*

yecorana Henrickson, Fishbein, & Van Devender, *Menodora yecorana* Van Devender & Turner, *Pectis vandevenderi* B.L. Turner, *Pinus yecorensis* Debreczy & Rácz, *Portulaca yecorensis* Henrickson & Van Devender and *Tridax yecorana* B.L. Turner, to mention but a few.

Adenophyllum yecoranum is a very distinctive species, though clearly relating to the *A. porophyllum* complex as conceived by Strother (1969). In the latter's seminal treatment of *Dyssodia porophylla* (= **Adenophyllum porophyllum**), the novelty will not key to any of his infraspecific taxa, having a unique combination of characters, as noted in the above diagnosis. In my treatment of *Dyssodia* of Mexico (Turner 1996), it will key to *D. cancellata* (= **Adenophyllum cancellatum**), this recognized as but a variety of **A. porophyllum** by Strother (1986).

The following key should serve to identify species within the **A. porophyllum** complex, as currently understood:

- 1. Heads discoid..... **A. porophyllum**
- 1. Heads radiate.....(2)
- 2. Ray florets yellow; ligules 6-8 mm long, 2-3 mm wide; pappus w/o an outer series of short scales; Son, nw Chi..... **A. yecoranum**
- 2. Ray florets orange; ligules 8-10 mm long, 6-7 mm wide; pappus with an outer series of short scales; wide spread.....**A. cancellatum**

ADENOPHYLLUM CANCELLATUM (Cass.) Villarreal, Acta Bot. Mex. 56: 10. 2001.

Adenophyllum porophyllum var. *cancellatum* (Cass.) Strother

Dyssodia cancellata (Cass.) A. Gray

Dyssodia fimbriata M.E. Jones

Dyssodia porophylla Willd., not *Dyssodia porophylla* (Cav.) Cav.

Dyssodia porophyllum var. *cancellata* (Cass.) Strother

Lebetina cancellata Cass.

Tagetes cancellatus (Cass.) Maza

Chi, Tam, Sin, Dur, Zac, Agu, San, Gua, Que, Hid, Nay, Jal and Mic, Central Plateau, mostly 1500-2000 m; Aug-Dec. **Map 1**

Strother (1969) included this taxon within his concept of **A. porophyllum** but, as noted under the latter, I do not accept such treatment, nor did Villarreal, who also accepted its specific status.

ADENOPHYLLUM POROPHYLLUM (Cav.) Hemsl., Biol. Cen. Amer. Bot. 2: 218. 1881.

Adenophyllum porophyllum var. *radiatum* (DC.) Strother

Boebera alternifolia Moc. & Sesse ex DC.

Dyssodia porophylla (Cav.) Cav.

Dyssodia porophylla var. *discoidea* DC.

Dyssodia porophylla var. *radiata* (DC.) Strother

Pteronia porophyllum Cav.

Son, Sin, Col, Gua, Jal, Nay, Mic, Mex, Mor, Pue, Ver, Gue, Oax, Cps, Yuc and Guatemala southwards, also the Caribbean regions, mostly tropical deciduous forests, 10-1500 m; Aug-Nov. **Map 2**

This species occurs mostly along the Pacific slopes and occasionally somewhat inland where perhaps introduced; it is also found along the Gulf slopes from s Ver to Cam. Strother (1969, 1986) distinguished a var. *radiatum* but I believe this to be but a form of var. *porophyllum* with reduced ray florets, this also suggested, indirectly, by Williams (1976).

McVaugh (1984) notes that in Jal, **Adenophyllum porophyllum** and **A. cancellatum** (treated by him as varieties) "sometimes occur together" but adds, "they differ rather strikingly" by a number "of subtle features of flowers and involucre." In addition to the eradiate heads in **A. porophyllum**, he notes that in young heads of the latter the disk florets are hidden by the pappus bristles and that the reverse condition holds for **A. cancellatum**. This appears to be a valid observation, and their co-occurrence without clear intermediates suggests that 2 species are involved, consequently I treat these as good, partially sympatric, taxa which do not interbreed, or rarely so.

Adenophyllum porophyllum also occurs near **A. yecoranum** in Sonora (ca 15 km N of Yecora, *Van Devender 98-1662* [TEX]), but there is no suggestion that the two taxa might hybridize.

ACKNOWLEDGEMENTS

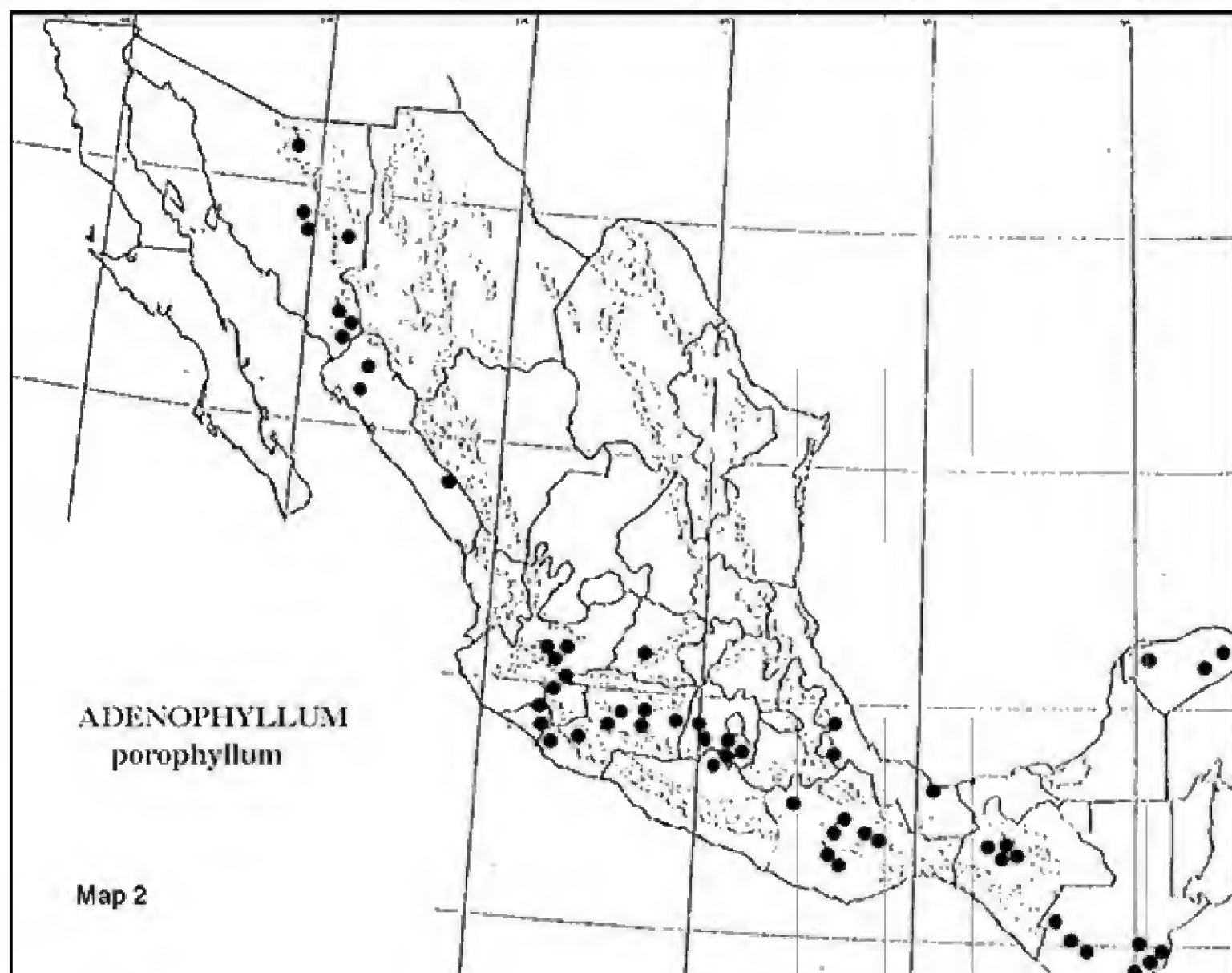
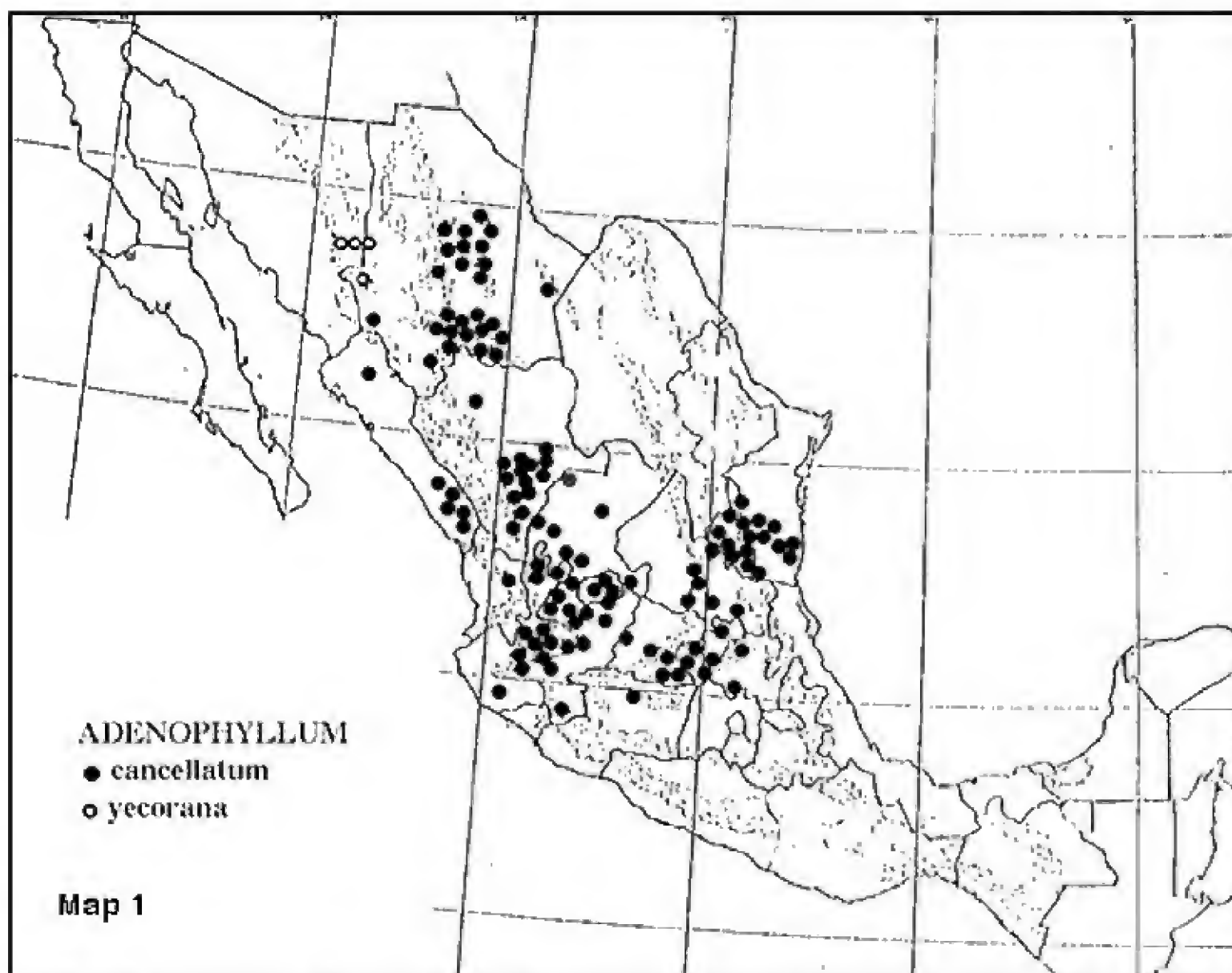
I am grateful to the input of Tom Van Devender for additional habitat data for the type locality, for pointing out the array of endemics in the Yecora area, and for calling to my attention specimens of the novelty collected by yet others. Jana Kos, my editorial assistant, provided helpful suggestions.

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Figure 1. Holotype of *Adenophyllum yecoranum*.



Bidens serboana* (Asteraceae: Coreopsideae), a new species from Oaxaca, Mexico*Billie L. Turner**

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ABSTRACT

A novel taxon, ***Bidens serboana*** B.L. Turner, **sp. nov.**, is described from Distrito Yautepec, Oaxaca, Mexico. It is presumably closely related to *Bidens minensis* but differs in numerous characters, including rayless heads and broad, imparipinnate, glabrous leaves. Published on-line: www.phytologia.org *Phytologia* 95(1): 23-26 (Feb. 1, 2013).

KEY WORDS: Asteraceae, Coreopsideae, *Bidens minensis*, *Bidens odorata*, Mexico, Oaxaca

Preoccupation with the Comps of Mexico (cf. Turner 2010, etc.) has occasioned the present paper.

BIDENS SERBOANA B.L. Turner, **sp. nov.** **Fig. 1**

Resembling *Bidens minensis* Sherff in habit but having eradiate heads (vs white-rayed); more numerous disc florets (20-30 vs ca 15); larger leaves (8-11 cm long, vs 1-4 cm); and more coarsely ciliate, larger, achenes (20-30 mm long vs 10-12 mm).

Annual herbs (?), to 50 cm high. **Stems** (mid-section), 4-sided, minutely pubescent along the angles, ca 1.5 mm thick. **Leaves** (mid-stem), 8-12 cm long, 6-8 cm wide, tripartite, the segments thin and glabrous, mostly 1-4 cm long, 5-6 mm wide; petioles 2-4 cm long. **Capitulescences** arranged both axillary and terminal, the latter divaricately branched, ca 10 cm high, 20 cm across; ultimate peduncles 4-6 cm long, minutely pubescent with upturned hairs. **Involucral bracts** (outer) 8, linear-oblongate, 4-5 mm long; inner bracts 8, broadly lanceolate, ca 5 mm long. **Receptacular bracts** 4-6 mm long, linear-lanceolate, glabrous, brown on the dorsal surfaces. **Ray florets**, absent. **Disc florets** 20-40; corollas yellow, ca 3.5 mm long, glabrous. **Stamens** brown. **Achenes** (interior) 6-20 mm long, ca 1 mm wide, radially flattened, mostly glabrous, except for the margins which possess stiffly, up-curved hairs, with markedly swollen bases, the exterior achenes ciliate both marginally and dorsally, these grading into those of the interior.

TYPE: MEXICO: OAXACA. Distrito Yautepec, Mpio. San Bartolo Yautepec, “El Palacio. Sabana. plano, suelo pedregoso.” 16 27 57.9 N, 95 58 0.3 W, ca 1559 m, 20/10/2011, *Dionisio Lopez Pascual* (DIL) 1606 (Holotype: TEX)

When first examined, I took this novelty to be closely clearly related to *Bidens odorata* var. *oaxacensis* Ballard, since it keyed to or near that taxon in the treatment of *Bidens* by Melchert (2010). However, it possesses a combination of characters unlike anything known in the *B. odorata* complex, namely, rayless heads and large, markedly tripartite, glabrous leaves with broad segments. Indeed, among the 60 or more specimens of var. *oaxacensis* housed in the LL-TEX herbarium, not a single sheet can be found that comes close to the novelty proposed herein, nor can rayless plants be found among them, not to mention the very distinct foliage.

Melchert, upon reading the above comments, suggested that the novelty might be more closely related to the poorly known **B. minensis** Sherff, this known only by the type (from Distrito Mina, Gue) and a single collection from 6 mi N of Taxco, Gue (*Rzedowski 2517*, TEX). My comparisons of the

collections concerned suggest that his observations are valid, hence my comments in the above diagnosis. Fig. 2 shows the distribution of **B. minensis** and the newly described **B. serboana**.

The species name is an acronym of the Sociedad para el Estudio de los Recursos Bioticos de Oaxaca (SERBO). This organization has helped fund the collection of numerous plants from the area concerned.

ACKNOWLEDGEMENTS

I am grateful to Jana Kos and my Academic son, Prof. Emer. Thomas Melchert of the Univ. of Iowa, for reading the manuscript and providing helpful comments. The distribution map (Fig. 2) is based upon specimens on file at LL-TEX.

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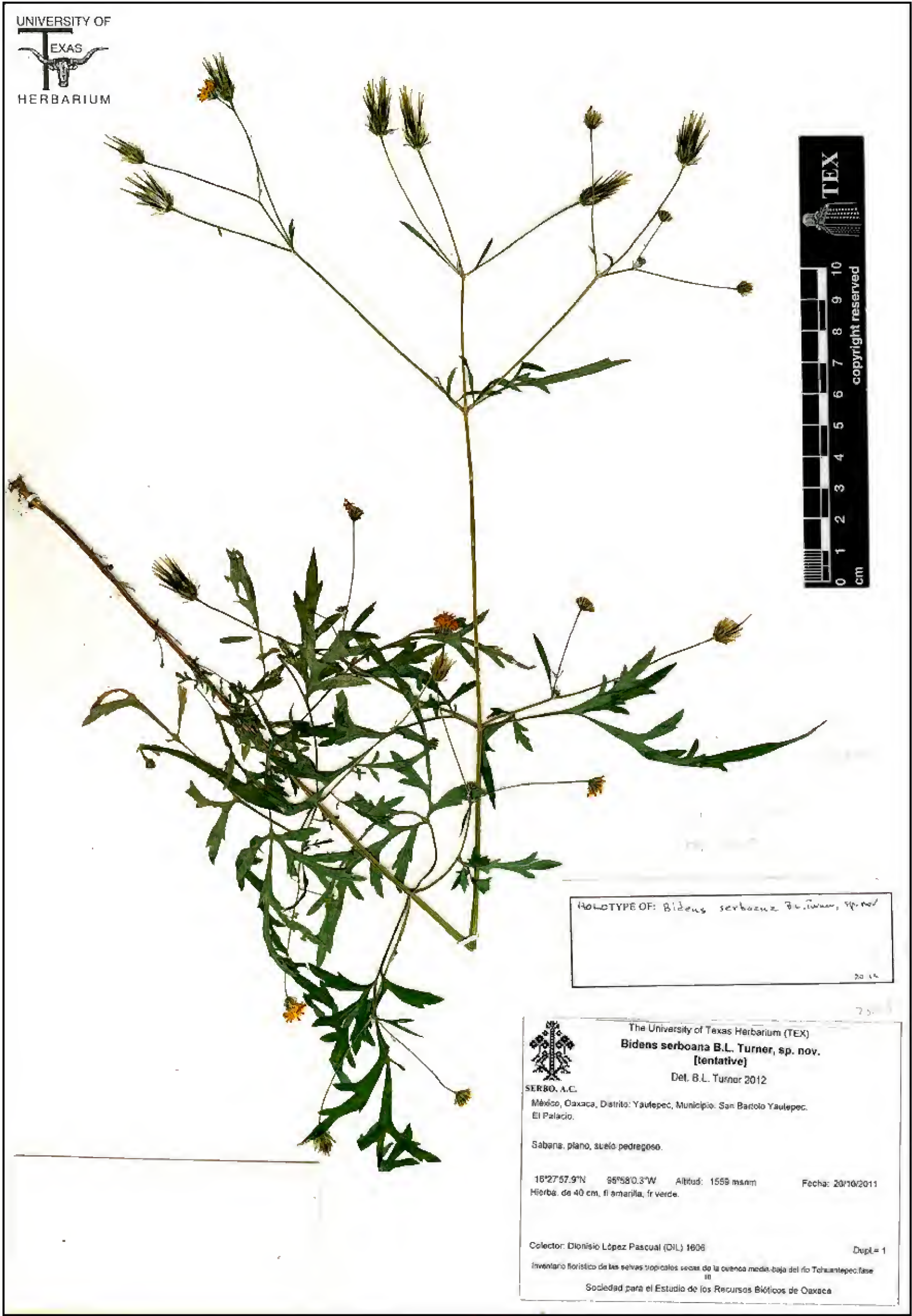


Fig. 1. *Bidens serboana* (Holotype: TEX).

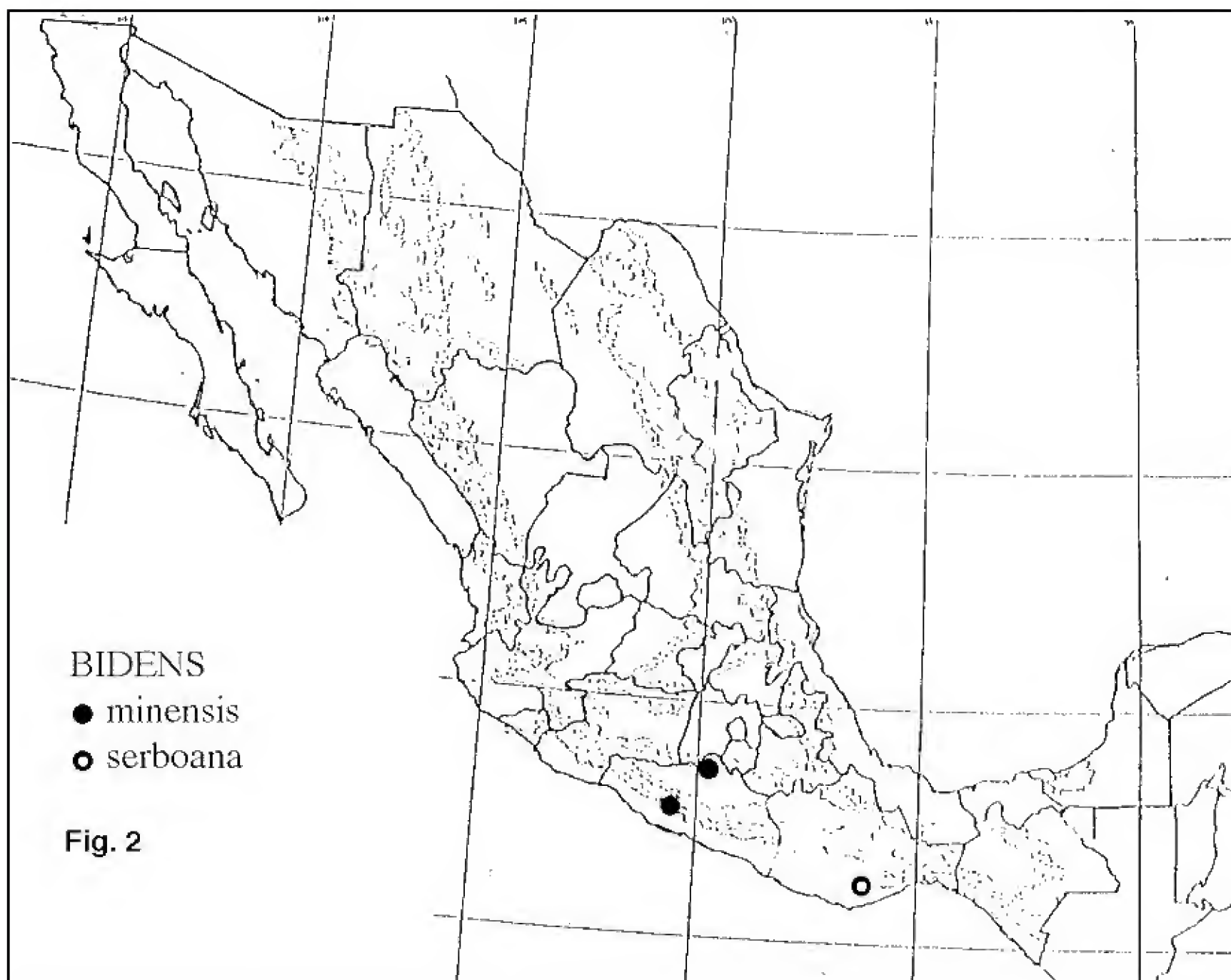


Fig. 2. Distribution of ***Bidens minensis*** and ***B. serboana***.

Mexican species of *Stachys* (Lamiaceae) revisited

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ABSTRACT

In my synoptical study of the Mexican and Central American *Stachys* (Turner 1994b), 38 species were recognized; two species were added by subsequent workers, *S. manantlanensis* B.L. Turner and *S. turneri* Rzed. & Calderon, these discussed herein. An additional two novelties are described from Mexico in the present paper: *Stachys tamaulipana* B.L. Turner, **sp. nov.**, from the states of Tamaulipas and Nuevo Leon, and *Stachys tlaxiacana* B.L. Turner, **sp. nov.**, from the state of Oaxaca; the former relates to the more northern *S. boraginoides*, the latter to the more southern *S. grahamii*; photographs of the holotypes are presented, along with maps showing their distribution.

Published on-line: www.phytologia.org *Phytologia* 95(1): 27-33 (Feb. 1, 2013).

KEY WORDS: Lamiaceae, *Stachys*, *S. manantlanensis*, *S. turneri*, Mexico, Oaxaca, Nuevo Leon, Tamaulipas

As noted in the above abstract, two novelties were added to the Mexican species of *Stachys* following my synoptical treatment of the complex. Appropriate comments upon their status follow:

STACHYS MANANTLANENSIS B.L. Turner, *Phytologia* 78: 209. 1995.

As noted in the original description, this taxon belongs to the *S. coccinea* complex (Turner 1994a). In my synoptic treatment of the Mexican species of *Stachys* (Turner 1994a), it will key to *S. pacifica* B.L. Turner, but differs in habit (sprawling stems, rooting at the nodes) and corolla color, which was said to be “bright magenta to lilac” in my original description; at the time the following two collections were not available to me: *Ilitis* 31124 (TEX), who described the corollas as “deep rose,” while *Ilitis et al.* 29354 (TEX) describe the corollas as a “rich deep rose.”

STACHYS TURNERI Rzed. & Calderon, *Acta Bot. Mexicana* 32: 4. 1995.

As noted by its authors, this is a very distinct taxon, presumably confined to the state of Guanajuato, and presumably closely related to *S. arriagana* B.L. Turner and *S. moorei* B.L. Turner, all possessing pubescent nutlets, a relatively rare trait in the genus. The following key will distinguish the taxa concerned:

1. Stems and foliage densely pubescent with white villous hairs;
corolla tube annulate; Guanajuato.....**S. turneri**
1. Stems and foliage otherwise; corolla tubes lacking an annulus;
Hidalgo and San Luis Potosi...(2)
2. Calyx 6-7 mm long; corolla tubes pink, 5-9 mm long;
Hidalgo.....**S. moorei**
2. Calyx 9-10 mm long, corolla tubes lilac, 10-11 mm long; San Luis
Potosi.....**S. arriagana**

It should be noted, ashamedly, that in my synopsis of Mexican taxa (Turner 1945b), I keyed both *S. arriagana* and *S. moorei* as possessing annulate corolla tubes, although these were appropriately described as lacking an annulus in my original descriptions of the taxa. Fortunately, the authors of *S. turneri* correctly noted the error concerned.

Distribution of the several taxa is shown in Fig. 3.

My continued interest in the Mexican *Stachys* has revealed two additional novelties, as follows:

STACHYS TAMAULIPANA B.L. Turner, **sp. nov.** Fig. 1

Rhizomatous perennials, rooting at the nodes and forming large mats up to 30 cm high in wet places. **Mid-stems** pubescent with spreading hairs 1.5-2.0 mm long, beneath these, on uppermost stems, an array of minute, glandular, hairs. **Leaves** (at mid-stem) mostly 5-10 cm long, 3-4 cm wide; petioles 2-6 cm long; blades sub-cordate to cordate, sparsely pubescent above and below with hairs 1-2 mm long; margins with rounded serrations. **Inflorescence** a terminal bracteate raceme, 10-20 cm long; peduncles 5-15 cm long; bracts leaf-like, lanceolate, reflexed, 1-4 cm long, 0.2-1.0 cm wide. **Flowers** 4-6 to a node; pedicels 1-2 mm long, minutely, glandular-pubescent. **Calyces** (flowering) 5-6 mm long, minutely pubescent to nearly glabrous, the lobes lanceolate, 2-3 mm long. **Corollas** reportedly “pink (*Hinton 24613*)” or “deep purple (*Ferguson 7*);” tubes 6-8 mm long, having a well-defined annulus ca 3 mm from the base; lower lip 6-8 mm long; upper lip 3-4 mm long. **Anthers** purple, extending from the throat for ca 3 mm. **Nutlets** brown, 1.5-2.0 mm long, verrucose or somewhat warty (not smooth).

TYPE: MEXICO. TAMAULIPAS: Mpio. Hidalgo, “Arroyo Obscuro; along road to Dulces Nombres, Nuevo Leon; 2.0 road miles NE of Los Caballos towards Canada El Mimbres; 15.0 road mi from the lowermost crossing of arroyo El Mimbres; humid forest with *Carya* [et al.],” limestone soils, 1800 m, 23 59 09 N, 99 28 37 W, “forming extensive mats in wet rocks of intermittent stream,” 23 Sep 1994, *Mark H. Mayfield 2086* [with J. Hinton & G. Nesom] (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. NUEVO LEON; “CO/MPIO: cadereyta; 1.2 km SW of the junction with the main road from Cadereyta to Allende towards Santiago along the road through La Boca Canyon to Antiago in the Sierra La Silla in low lying areas; east of the Sierra La Silla;” occurring with *Taxodium*. “Dark clay soils. Common rhizomatous perennials; growing in roadside ditches; corolla deep purple.” 350 m, 14 Mar 1994, *Ferguson 7* (TEX). **TAMAULIPAS: Mpio. Hidalgo**, Los Caballos, 1700 m, 3 Aug 1994, *Hinton et al. 24613* (TEX).

The species is named for the state of Tamaulipas, whence the type.

In my treatment of Mexican *Stachys* (Turner 1994b), this species, because it lacks broad-based stem-hairs, will key to *S. pilosissima* Mart. & Gal.; at the time of that treatment, I possessed only one collection of the novelty from Tamaulipas (*Hinton et al. 24613*), this I positioned in the latter taxon, lacking detailed descriptive data, etc. Subsequent collections (cited above) strongly suggest that the taxon is undescribed, and perhaps closer to *S. boraginoides* Schlecht. & Cham., having a sprawling habit and relatively large annulate corollas, as well as large, somewhat verrucose, nutlets. While treated as a novelty here, it must be admitted that the taxon might with equal validity be treated as part of the fabric of an enlarged *S. boraginoides*. It differs from the latter, however, in several features, including vestiture (lack of broad-based hairs), lanceolate, reflexed flowering bracts (vs leaf-like and non-reflexed), shorter calyx lobes (2-3 mm long vs 3-5 mm), and distribution (Fig. 4).

STACHYS TLAXIACANA B.L. Turner, **sp. nov.** Fig. 2

Rhizomatous, erect, perennial herbs to 30 cm high. **Mid-stems**, mostly glandular-pubescent (setulose and eglandular near the base), the vestiture 0.3-0.5 mm high. **Leaves** (lower), 2-3 cm long, 1.0-1.5 cm wide; petioles 1-6 mm long; blades broadly lanceolate to sub-deltoid, appressed-pubescent above and below, the margins minutely serrate. **Inflorescence** a terminal interrupted, glandular-pubescent, spike ca 18 cm long, 3 cm wide; floral bracts broadly obovate, 3-5 mm long and as wide, glandular-pubescent mainly along the margins, their surfaces appressed-pubescent. **Flowers**, 4-6 to a node, the internodes ca 2 cm long. **Calyces** (flowering) 4-5 mm long, pubescent like the bracts; tubes ca 3 mm long, the lobes 1.5-

2.0 mm long. **Corollas** reportedly “purple;” tubes 7-8 mm long, having a well-defined annulus ca 2 mm above the base; upper lip ca 2 mm long; lower lip 3-5 mm long. **Anthers** purple, excurrent for ca 2 mm. **Nutlets**, smooth, brown, ovoid, ca 1.5 mm long, 1.0 mm wide.

TYPE: **MEXICO. OAXACA. Distrito Tlaxiaco**, “ca 10 mi N of San Miguel El Grande Slopes of Cerro Piedra de Olla. Pine, fir, on steep slopes and ridge[sic]. Rare in part shade under trees.” 2950 m, 97 33 W, 17 07 N, 3 Aug 1990, *J. A Soule* 2435 [with D.R.Brunner] (Holotype: TEX).

Stachys tlaxiacana will key to or near **S. grahamii** in the treatment of Turner (1994b); in addition to its distribution (Fig 5), it differs from the latter in being a stiffly erect small herb with glandular-pubescent stems (vs not so), having notably short calyx lobes (1.5-2.0 mm long vs 2-4 mm); especially noteworthy are the smaller, broadly obovate, glandular-pubescent floral bracts, such not found in **S. grahamii**.

The species name is derived from the Distrito Tlaxiaco, whence the type.

ACKNOWLEDGEMENTS

My field companion, Jana Kos, edited the paper. Maps are based upon specimens on file at LL-TEX, and those included in the work of Turner (1994a,b).

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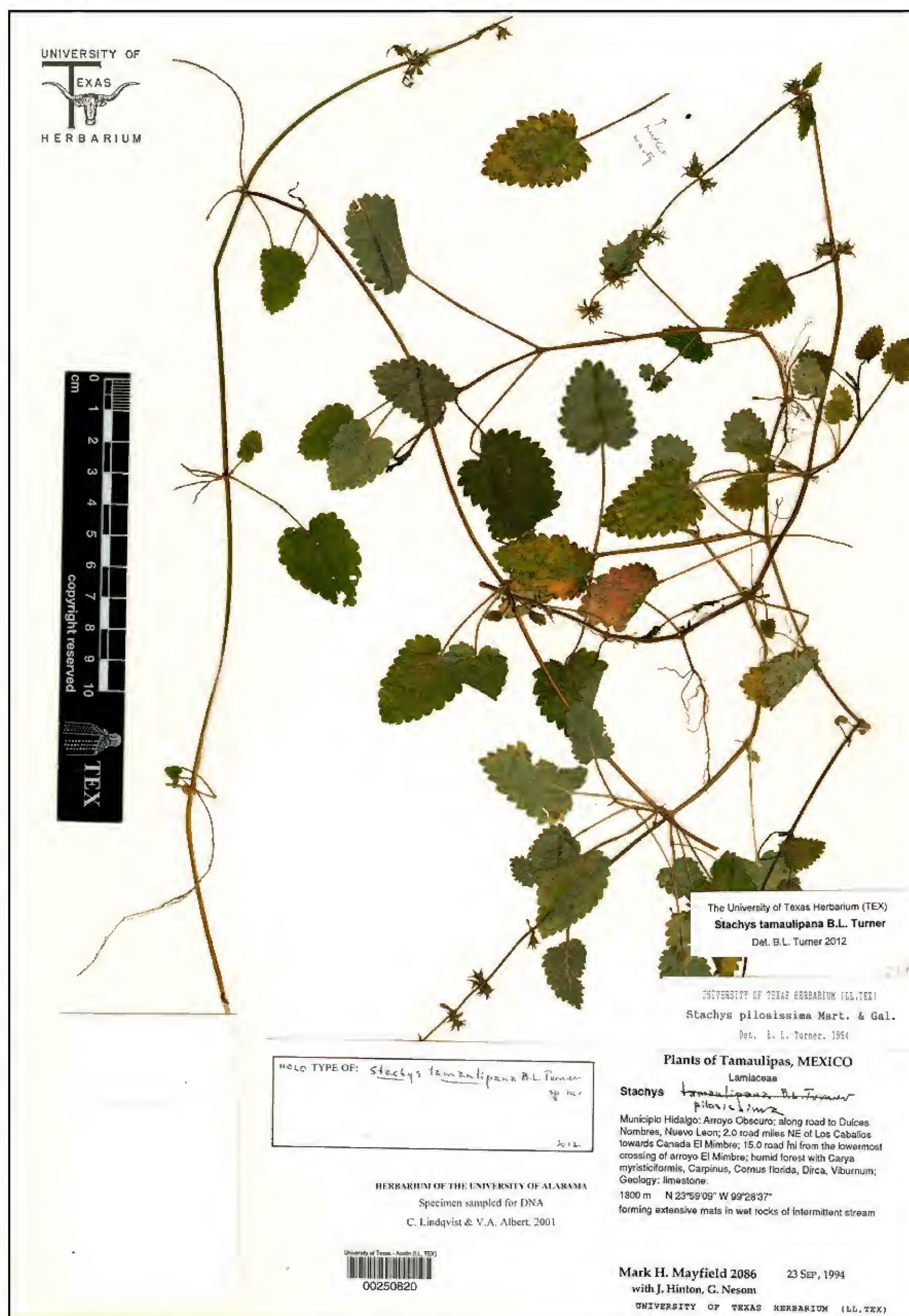


Fig. 1. Holotype of *Stachys tamaulipana* B.L. Turner (Holotype, TEX).

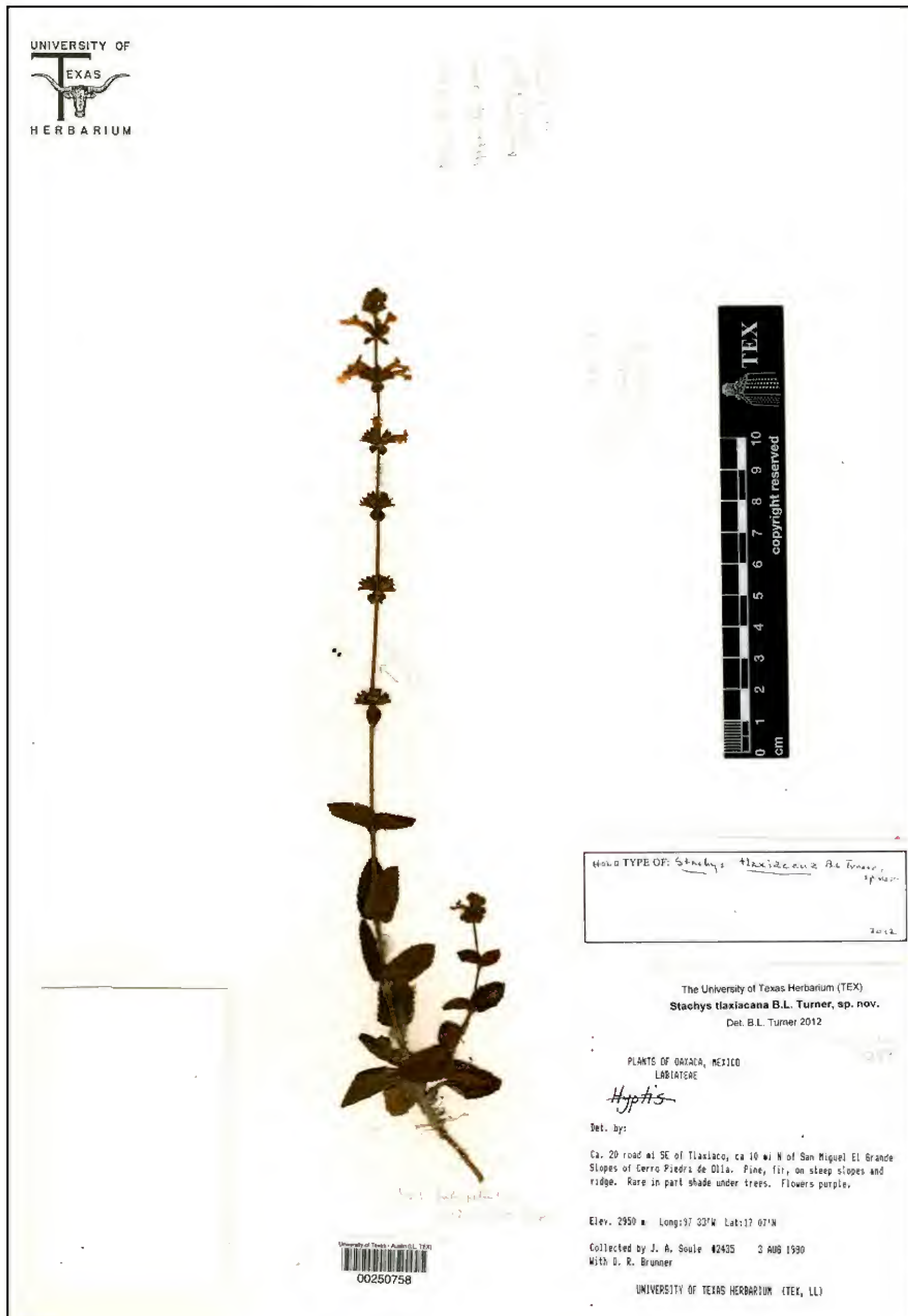


Fig. 2. Holotype of *Stachys tlaxiacana* B.L. Turner (Holotype, TEX).

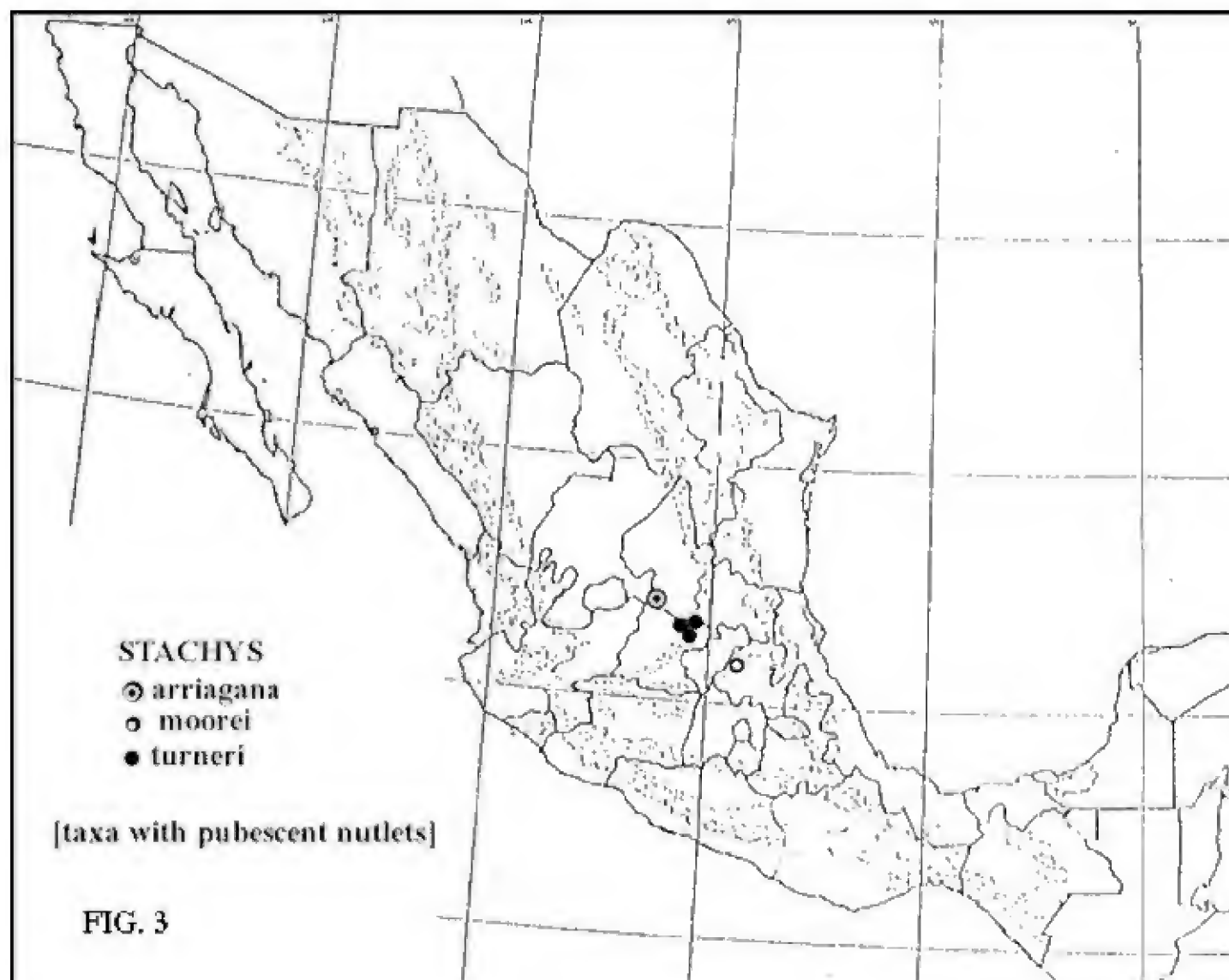


Fig. 3. Distribution of *Stachys turneri* complex.

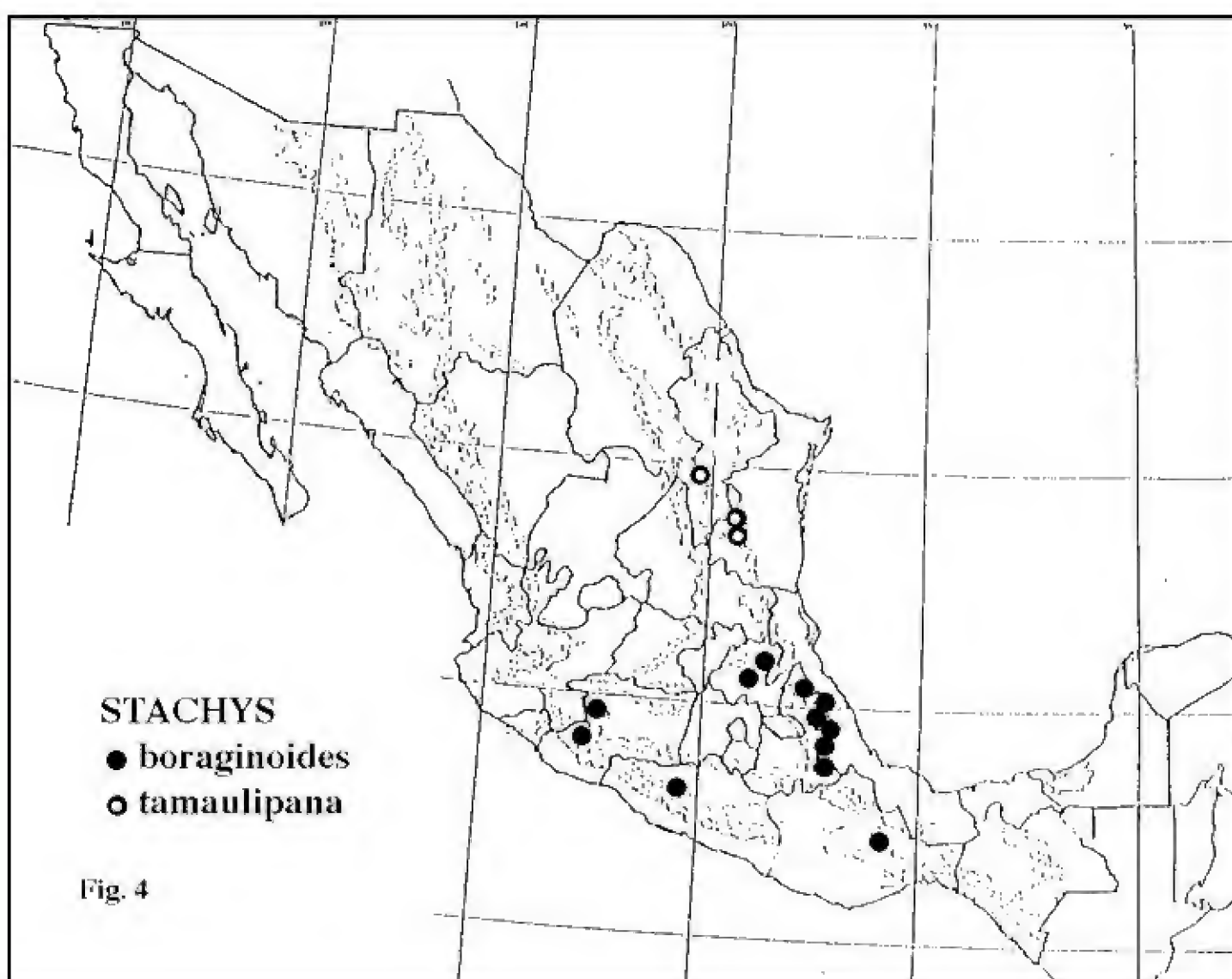


Fig. 4. Distribution of *Stachys tamaulipana* and *S. boraginoides*.

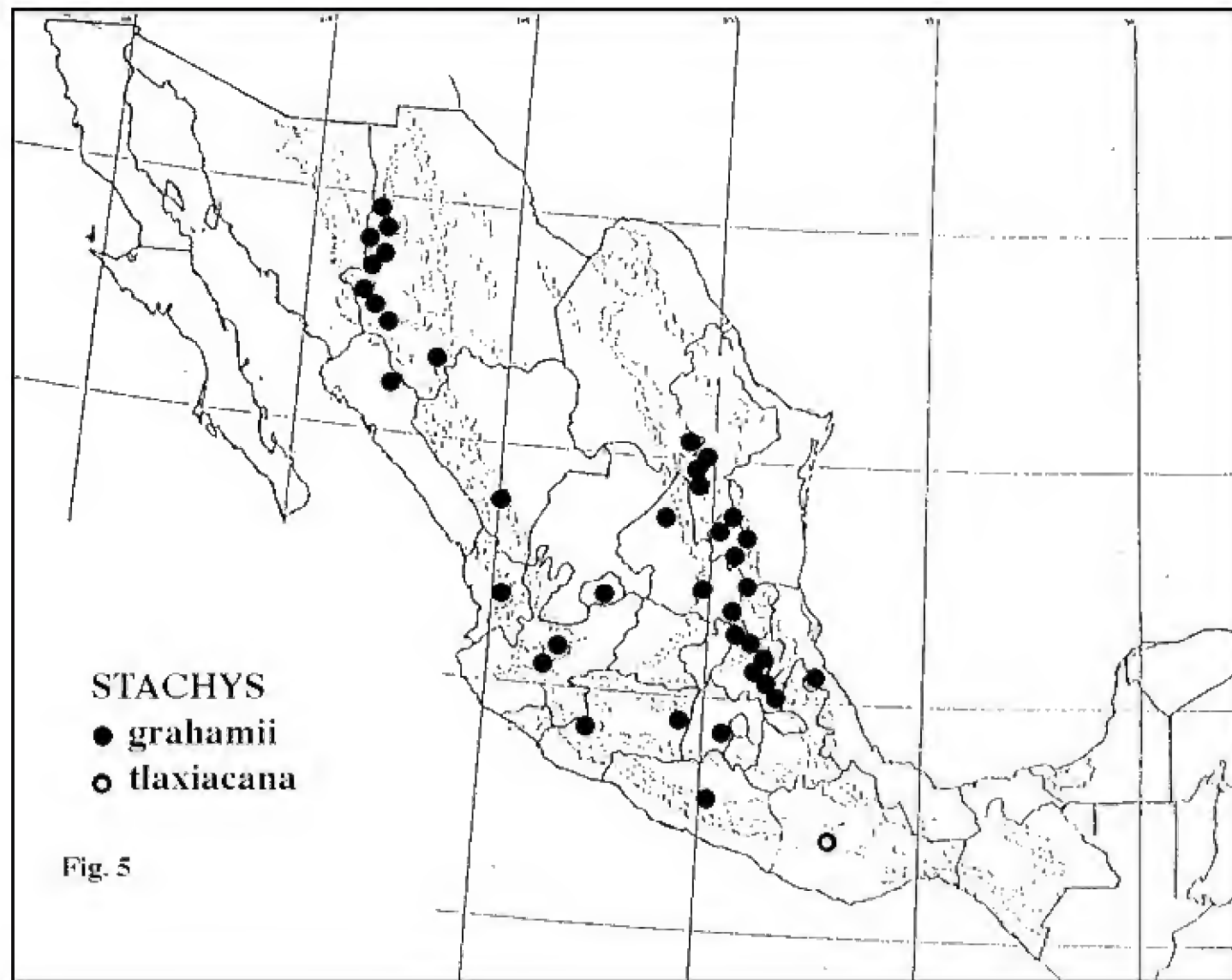


Fig. 5. Distribution of *Stachys tlaxiacana* and *S. grahamii*.

An oomycete parasitizing algae occurring on dorsal shells of turtles

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ABSTRACT

This paper reports the identity of a parasite (belonging to the Oomycota—and more broadly, the Straminipila), found in cladophoraceous algae (*Basycladia*) growing in tufts or more extensive coverings on the external surface of the carapace of freshwater turtles collected in Alabama and Mississippi. Whereas this Oomycete might have been placed in genus *Lagenidium* (historically a large group of plant and animal parasites), investigation by various workers has shown *Lagenidium* to be nomenclaturally (and systematically) questionable—its identity interwoven with a related (and also problematic) genus *Myzocyttium*. We do not attempt to resolve the nomenclatural origins of *Lagenidium* and *Myzocyttium*, but rather, after discussion of taxonomic problems, focus on placement of this Oomycete within current segregate genera (of *Lagenidium*)—established by Dick (1997, 2001), partly in response to taxonomic confusion. The best candidate for assignment of this Oomycete is Dick's genus *Syzygangia*. Since there is also some question about the particular species of the algal host (genus *Basycladia*), occurring on turtles we examined, the specific identity of the alga is given consideration herein as well. Published on-line: www.phytologia.org *Phytologia* 95(1): 34-41(Feb. 1, 2013).

KEY WORDS: *Basycladia*, *Lagenidium*, *Myzocyttium*, nomenclature, *Pythium*, *Sternotherus*, *Syzygangia*, *Trachemys*, type, zoosporogenesis.

A number of relatively inconspicuous, oomycetous pseudofungi—internal parasites of plants, other Oomycota, various invertebrates, etc.—are interesting subjects of study. Some are highly significant, well-studied plant pathogens, including potentially devastating forms such as late-blight of potato (*Phytophthora infestans*). Others, especially those with holocarpic life cycles (in which the “vegetative” thallus is converted, at a certain stage, entirely to reproductive structures), have often remained more poorly known (cf. Blackwell, 2011). The putatively holocarpic, Oomycete parasite considered here was found in algae (WB#86, October, 1998) attached to the external surface of the dorsal part (carapace) of the shell of turtles (inhabiting sloughs, creeks, or lake margins) in central Alabama—Stink-pot turtles (genus *Sternotherus*), Tuscaloosa Co., collected by Dr. Gordon Ultsch—and, in western Mississippi—harvested from “slider” turtles (genus *Trachemys*) collected in an area of catfish-farm-ponds near Itta Bena, MS, May 5, 2012, by Mr. Andrew DeSantis. The algal host (of the Oomycete) in both cases proved to be the green alga *Basycladia* (Cladophoraceae). This alga, though the subject of study, is incompletely known; we devote space to its history and species—as such relates to specimens in our study. The species identity of these algal specimens proved somewhat debatable. The Oomycete would formerly have been placed in the well-known (if not well-understood) genus *Lagenidium*. However, *Lagenidium* is fraught with nomenclatural problems, stemming from an imprecise original description and inadequate initial species designations under this generic name. Further problems arose from early confusion of *Lagenidium* and another lagenidiaceous genus, *Myzocyttium*. Morphological intergradation was noted between *Lagenidium* and *Myzocyttium* (Barron, 1976), and questions of relationship of included taxa persist. It became necessary to seek further generic placement for the Oomycete in our study.

HISTORICAL BACKGROUND OF *LAGENIDIUM*

The name *Lagenidium* was proposed by Schenk; however, the exact date of Schenk's publication (usually cited as 1859, cf. Sparrow, 1960) is debatable, since one or more parts of the publication may have appeared prior to 1859 (personal communication, Dr. Scott Redhead, Curator of the National

Mycology Herbarium, Canada), viz. 1858 (or possibly 1857). Schenk did not formally include species (binomials) in *Lagenidium*, though he suggested two very similar species, “*Pythium proliferum*” and “*Pythium globosum*”—names apparently coined by Schenk (cf. Matthews, 1931)—for possible inclusion. Only vegetative and asexual reproductive features of these two “taxa” were observed by Schenk. In complication, *P. proliferum* was also proposed by Schenk (1858), in a different publication, as the basis of his genus *Myzocyttium*, but no nomenclatural combination was provided there either. It is unclear if *Myzocyttium* was published before or after *Lagenidium* (given the uncertainty of the publication date of *Lagenidium*).

Walz (1870) treated *Pythium globosum* as a synonym of *P. proliferum*. Walz, however, appeared to be dealing with a mixed collection (cf. his figs. 13-19), i.e., what probably constituted *Pythium proliferum* (*sensu* Schenk) and *Lagenidium rabenhorstii* (Zopf, 1878)—as noted by Matthews (1931) and Dr. Readhead (personal communication). Regardless, the two “Pythiums” of Schenk (1859) are probably the same species; they do not in any case belong to *Pythium* (Matthews, 1931), but would be morphologically associated with either *Myzocyttium* or *Lagenidium*—depending on which generic name might have priority and whether either holds up as a legitimate name. Additionally complicating is that *L. rabenhorstii* Zopf, a different organism from the two “Pythiums,” has been considered (Scherff, 1925) the type of *Lagenidium*—a typification which Dick (2001) opposed, because *L. rabenhorstii* was not part of the concept of *Lagenidium* “established” by Schenk (1859).

Lagenidium taxonomy, already confusing, became complex, with a large (and diverse) number of species described—some, morphologically, seeming less closely related than others (cf. Sparrow, 1960; Karling, 1981). For a recent confirmation of “differential relatedness” of taxa (at one time or another placed in *Lagenidium*)—this involving molecular analysis—see Beakes and Sekimoto (2009).

“SOLUTION” OF THE “LAGENIDIUM PROBLEM”

No doubt in response to the frustrating state of *Lagenidium* systematics, Dick (2001), in a drastic step, excluded all species but one from the genus. More than 50 taxa were excluded by Dick and assigned to other genera, as species or synonyms. Approximately another 15 putative taxa of *Lagenidium* were considered “unidentifiable.” The only species retained by Dick (2001) in *Lagenidium* was *L. giganteum* Couch (1935)—a large and partly extramatrical form occurring on mosquito larvae. Initially (Couch, 1935), only asexual development was known for *L. giganteum*; a number years later, details of sexual development were described (Couch and Romney, 1973) but not illustrated. In any case, the morphological features of *L. giganteum* apparently suited Dick’s concept of what *Lagenidium* should represent. Dick (2001) proposed *L. giganteum* as “lectotype” of the genus. In seeming anticipation of excluding a number of species from *Lagenidium*, and some from *Myzocyttium*, Dick (1997) established several segregate genera, e.g., *Chlamydomyzium*, *Myzocytiopsis*, and *Syzygangia*. Regardless of his questionable typification of *Lagenidium* (not based on earlier material, such as in Zopf, 1878), and his severe alteration of *Lagenidium* taxonomy (and to an extent *Myzocyttium*), Dick’s segregate genera—in conjunction with lagenidiaceous genera still available (e.g., *Aphanomyces* Scherff), and the few taxa retained in *Myzocyttium*—can be viewed as at least a workable solution to difficult nomenclatural problems. We note, however, that some authors (e.g., Kiziewicz, 2004; Beakes and Sekimoto, 2009) continued to recognize certain species (though excluded by Dick, 2001) under the name *Lagenidium*.

At a minimum, Dick’s revised system (1997, 2001) has the benefit of allowing resolution of certain problems of relationship of organisms in the former Lagenidiaceae (cf. Karling, 1981). For example, Dick’s (1997) system served to clarify that traditional *Myzocyttium* housed, perhaps unnaturally, taxa which were either algal or animal (nematode and rotifer) parasites. This enabled Pereira and Vélez (2004) to properly assess that the algal parasite they observed (possessing extrasporangial zoosporogenesis, and catenulate thallus morphology)—described initially as *Myzocyttium megastomum* by

Wildeman (1893)—should be retained in *Myzocyttium*, and not placed in *Myzocytiopsis* (designated by Dick for animal parasites, and exhibiting intrasporangial zoosporogenesis). Conversely, recognition of Dick's (1997) genus *Myzocytiopsis* permitted proper referral of *Myzocyttium vermicolum* to *Myzocytiopsis*—in an ultrastructural study of this nematode parasite by Glockling and Beakes (2006).

TAXONOMIC PLACEMENT OF THE OOMYCETE PARASITE

Based on thallus morphology and nature of parasitism, the oomycetous algal parasite in our study (Figs. 1-5) would have been placed in *Lagenidium*—had problems with the nomenclature and taxonomy of this genus not been detected. Examination of classical treatments, e.g., Cook (1935), Sparrow (1960) and Karling (1981), reveals a range of algal-inhabiting *Lagenidium* species. In comparing traditional *Lagenidium* taxa with the Oomycete observed in *Basicladia* (the first report of an Oomycete parasitizing this genus that we are aware of) there are approximations, but no precise match. *Lagenidium marchalianum* Wildeman, occurring in the green alga *Oedogonium*, bears some resemblance, except that the tubes of its thallus are more slender and straight. *Lagenidium marchalianum* was transferred by Dick (1997) to *Syzygangia*, as *S. marchaliana*. The thallus of *Lagenidium oedogonii* Scherffel, a taxon also transferred by Dick to *Syzygangia*, is more delicate than in our parasite, and its “hyphae” pass readily from cell to cell of the host. Our parasite tends to be confined to individual host cells (though sometimes extensively developed within, cf. Figs. 2-4, even completely filling, the cell); it does not appear to as readily traverse the thick and often lamellated cross-walls (Figs. 6, 9) of *Basicladia*, although we have observed adjacent cell infection, and possible penetration of the end-wall (Fig. 4). *Lagenidium closterii* Wildeman, found in desmids, also somewhat resembles our parasite, but its thallal tubes are more slender, and sometimes—in addition to the usual intramatrix growth—develop extramatrix. *Lagenidium destruens* Sparrow, occurring as a parasite in the Saprolegniaceae genus, *Achlya*, can rather strongly resemble our organism, especially in potentially filling (and destroying the contents of) a host cell; however, the short, stubby, lobed (often “single-celled”) branches of *L. destruens* do not usually exhibit the more extensive, almost “mycelial,” development sometimes observed in the *Basicladia* parasite (Fig. 4); also, the (asexual) resting spores of *L. destruens* are more squarose or rectangular than the more spherical spores (Fig. 5) observed in the *Basicladia*-inhabiting organism. Zoospores were not observed, but germinating zoospore cysts were (Fig. 1). Though we found no precise comparison for the Oomycete in *Basicladia*, only asexual stages were seen. Nonetheless, an algal parasite such as this is best placed in genus *Syzygangia* (cf. Dick, 1997, 2001)—described for endoparasites of plants, especially algae. Generic referral seems clear, but this possibly undescribed taxon must be left for now as *Syzygangia* sp.

IDENTITY OF THE ALGAL HOST OF THIS PARASITE

Algae attached to dorsal shells of freshwater turtles are usually members of the Cladophoraceae (cf. Skinner et al., 2008), the primary such epizootic genus being *Basicladia*. The algal host in our study (Figs. 6-10) is identified as *Basicladia* (cf. Smith, 1950), reaffirmed as a genus distinct from *Cladophora* (see Garbary, 2010)—based not only on unusual habitats occupied, but on differentiation of the thallus into a branched prostrate-system and an upright-system of mostly unbranched filaments. Van den Hoek (1963), in his revision of European species of *Cladophora*—and with only brief reference to North American taxa—had tentatively viewed *Basicladia* as a Section of *Cladophora* (the grounds for such inclusion not being entirely clear). *Basicladia* in North America, as typically seen in freshwater environments, is unusual (among any kinds of algae) in preferential occurrence on the carapace of turtles (Fig. 10)—though it may be found, or cultured, on other hard (even inorganic) substrates (cf. Prescott, 1962; Graham et al., 2009); one species occurs on shells of snails (Normandin and Taft, 1959).

A total of seven species of *Basicladia* are known worldwide (Garbary, 2010; and *AlgaeBase*, see Guiry, 2012), but only three are native to the United States (two of these, *B. crassa* and *B. chelonum*, are found attached to turtle shells). *Basicladia*, as seen on turtles, is coarsely filamentous, and the basal-

system may have attachment or “holdfast” cells (Hoffman and Tilden, 1930). The often substantial algal covering on the dorsal shell has led to the nickname “moss back” for such inhabited turtles (Hoffmann and Tilden, 1930). Hoffmann and Tilden expressed surprise that algae as distinct as *Basicladia* were undescribed (as a genus) prior to 1930; though known earlier, they were misplaced in genus *Chaetomorpha* (noted by Normandin and Taft, 1959). *Basicladia* remained poorly known, and is not mentioned in every phycology text—e.g., Bold and Wynne (1985), even though they provided a treatment of Cladophoraceae. The genus, however, received recent recognition in Graham et al. (2009).

Turtle-inhabiting species of *Basicladia* in the United States occur mainly east of the Rocky Mountains—*B. crassa* is a northern species, and *B. chelonum* is more wide-spread (cf. Hoffmann and Tilden, 1930; Smith, 1950; Prescott, 1962). These species are distinguished by dimensions of vegetative cells—larger in *B. crassa*. Both were reported mainly on snapping turtles (genus *Chelydra*), cf. Prescott (1962) and Graham et al. (2009), but other turtle hosts were noted (Hoffmann and Tilden, 1930). Anderson and Sinclair (1966) studied *Basicladia crassa* collected from “western painted turtle,” “*Chrysemys picta bellii*,” in Illinois. *Basicladia* has been examined infrequently in the southeastern US; *B. chelonum* was reported in North Carolina (Whitford and Schumacher, 1969) “on several species of turtles, especially the common mud turtle” (identities now difficult to determine). In our study—central Alabama, western Mississippi—the alga was found on turtles identified, respectively, as “stink-pots” (*Sternotherus odoratus*) and “red-eared slider” (*Trachemys scripta elegans*). It is not surprising to find *Basicladia* on these turtles, given habits of shallow submersion. Neill and Allen (1954) noted that a number of kinds of turtles (including *Sternotherus odoratus*, and *Macrochelys temminckii*, the alligator snapping turtle) may be “epizoized” by algae, presumably *Basicladia*, in Florida. The closest fit for the *Basicladia* we found (similar in Alabama and Mississippi) is *B. chelonum*. But, cell-widths of our specimens (distal filaments)—typically in the 30 to 60µm range—overlap measurements for *B. chelonum* and *B. crassa* (Hoffmann and Tilden, 1930). Also, pre-zoosporogenesis germination papillae (Figs. 7-8) are more prominent than illustrated for either species. Thus, the alga (as well as the Oomycete) here considered could bear future scrutiny.

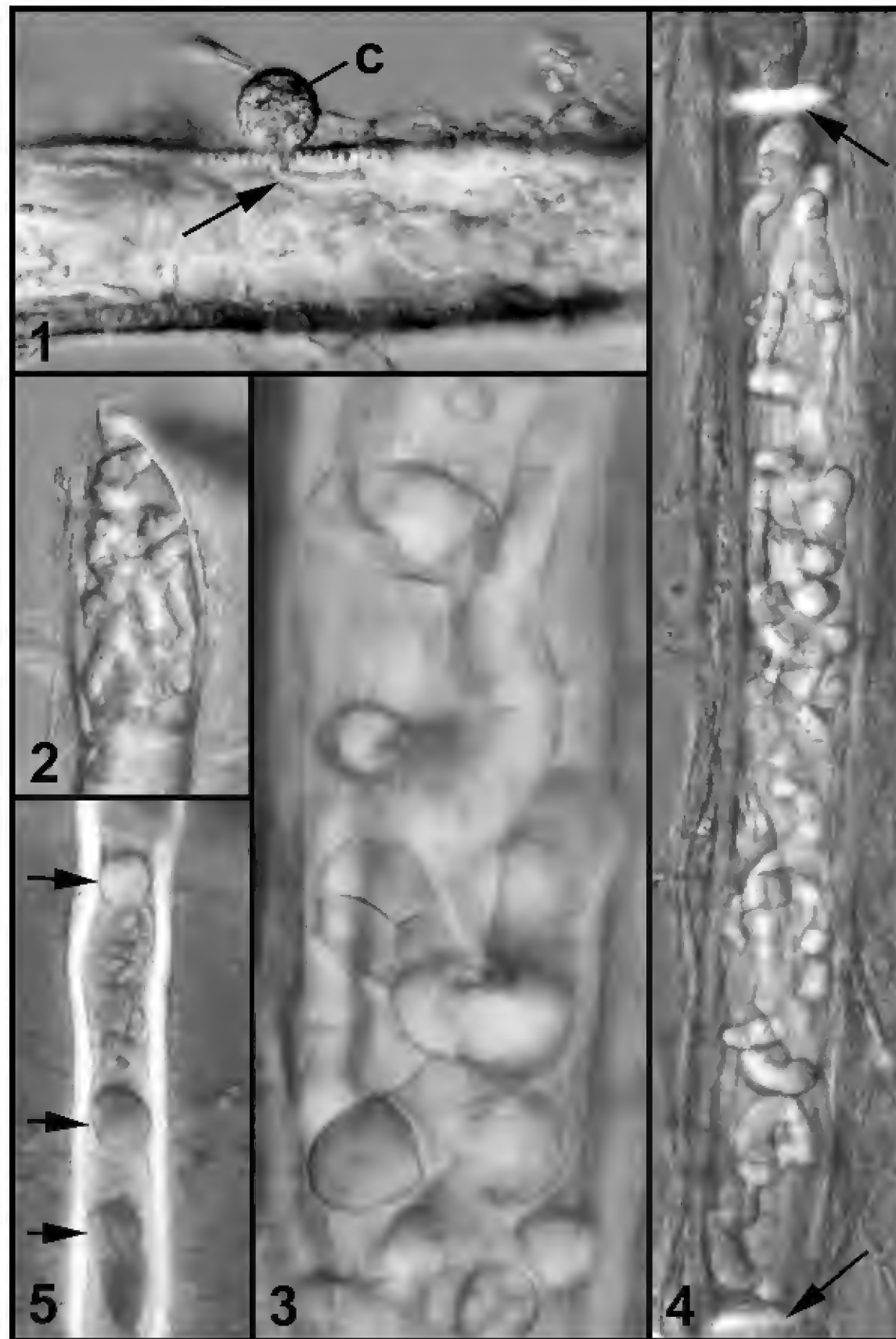
ACKNOWLEDGMENTS

We thank Drs. Robert Roberson (Arizona State University) and Sonali Roychoudhury (Patent Agent, New York) for manuscript review—and Dr. Stephen Secor (U. Alabama) for help with the project.

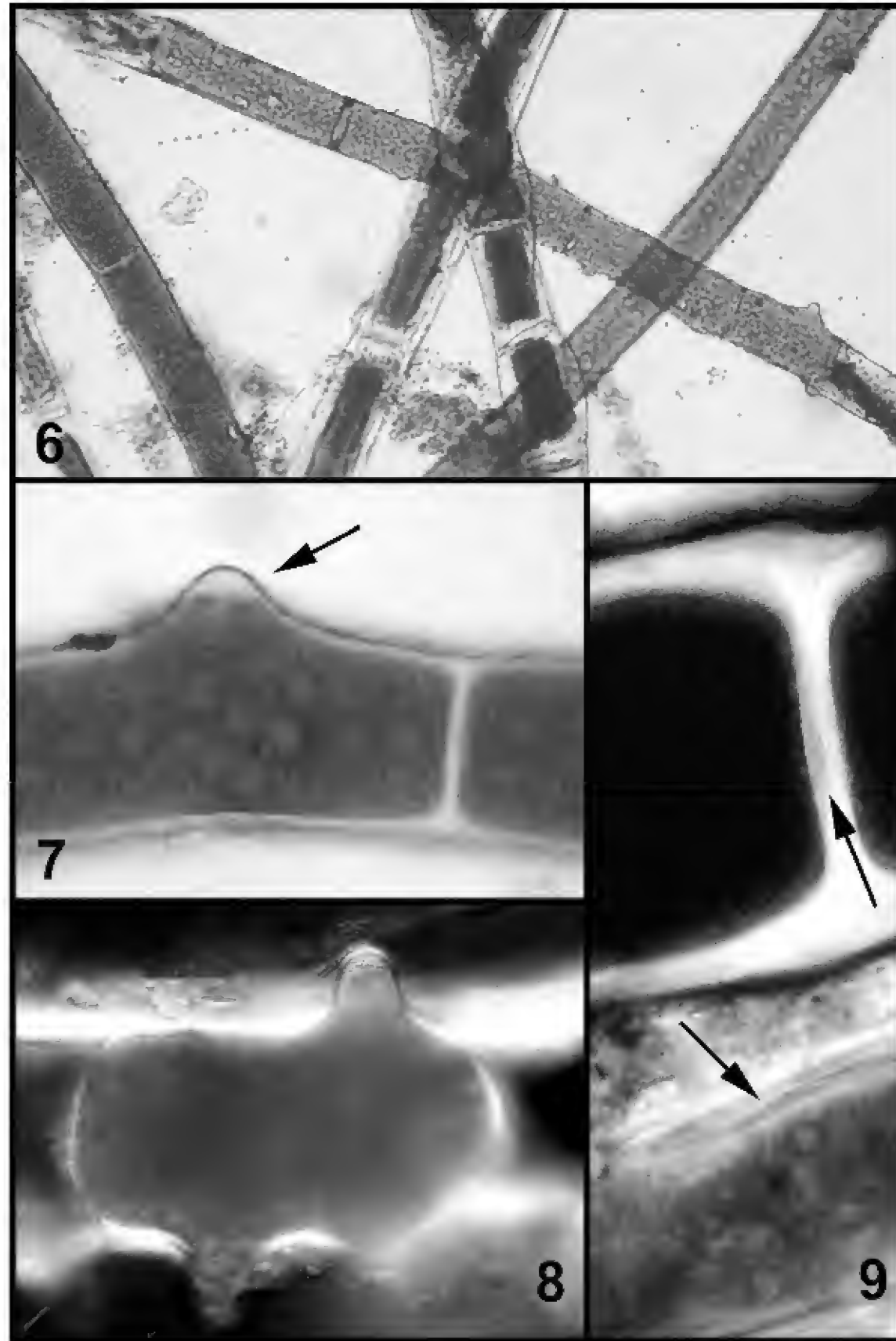
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Figures 1-5, *Syzygangia* sp. (Oomycota) in the green alga *Basicladia*. Fig.1: Zoospore cyst (C) of *Syzygangia*; germination tube development (arrow). Fig. 2: Vegetative thallus of *Syzygangia* in terminal cell of an algal filament. Fig. 3: Closer view of thallus in intercalary algal cell. Fig. 4: Extensive development of *Syzygangia* thallus from septum to septum (arrows) of an algal cell; possible penetration of thallus into adjacent algal cell, above upper septum. Fig. 5: Rounded *Syzygangia* resting spores (arrows), probably asexually formed.



Figures 6-9, *Basicladia* (similar to *B. chelonum*), Cladophoraceae. Fig. 6: General view of representative, non-parasitized, rather coarse and unbranched algal filaments; thickened cross-walls and germination bumps occasionally evident. Fig. 7: Closer view of pre-zoosporogenesis germination papilla (arrow). Fig. 8: Multiple, germination papillae on one cell. Fig. 9: Thickened, lamellated cell-walls evident: cross-wall (upper arrow), lateral wall (lower arrow).



Figure 10, “Stink-pot” turtle (*Sternotherus odoratus*). Raised areas on carapace (arrow) are temporarily dried algae (*Basicladia*).

Multivariate detection of hybridization using conifer terpenes II: Analysis of terpene inheritance patterns in *Pseudotsuga menziesii* F₁ hybrids

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ABSTRACT

The compositions of the volatile leaf oils of *Pseudotsuga menziesii* var. *menziesii* and var. *glauca* and their hybrids were examined and utilized to examine multi-variate methods for the detection of hybridization. The first cross (coastal parent 226 x inland parent 267) produced four hybrids whose oils were much like the inland (var. *glauca*) parent, and 6 hybrids with composition more intermediate between the parents. Eleven of the terpenes were generally intermediate in the hybrids, whereas the other 15 terpenes were transgressive. Truncation of the terpene values to those of the maximum or minimum value of the parents improved PCO ordination using character weights of Fs from ANOVA. A second set of hybrids between coastal parent 517 x intermediate parent 521 yielded similar kinds of variation in the terpenes of the hybrids. Only 2 compounds were intermediate between parents whereas 8 terpenoids showed dominance with values like one of the parents. Nine of the terpenes and percent oil yield were transgressive to both parents. Using F weighting and truncation of transgressives, improved the ordination separating parents from hybrids. However, again, the hybrids were ordinated in two groups. Selecting a balanced set of discriminating terpenes, aided in ordination. In a study of natural field hybridization, the terpenes should be useful to classify the hybrids, but it may be difficult to accurately classify back-crossed or F₂ individuals. Published on-line: www.phytologia.org *Phytologia* 95(1): 42-57 (Feb. 1, 2013).

KEY WORDS: *Pseudotsuga menziesii*, *P. m.* var. *glauca*, Douglas-fir, coastal, inland, hybrids, essential oil, terpenes, inheritance, genetics, multi-variate methods.

There are few studies on methods for the detection of hybridization using conifer terpenes from known crosses. The literature was recently reviewed (Adams and Tsumura, 2012). However, in general, Adams (1982) used leaf terpenoids to compare Wells' hybrid distance diagrams, PCA, PCO, and canonical variate analysis, but he had to use putative natural hybrids in *Juniperus*. He found that PCO, using character weighting of F-1 (F ratios from ANOVA between the putative parents), was the most effective method tested.

Confounding the problem is the fact that terpenes are inherited both as intermediate values (additive multi-gene quantitative) and as simple dominant recessive characters as well as transgressive values for many terpenes in a complex mixture (Adams and Tsumura, 2012).

Adams and Tsumura (2012) reported on the leaf volatile oils of two cultivars of *Cryptomeria japonica*, cv. Haava and cv. Kumotooshi, along with their 22 hybrids. The oil of Haava contains appreciable amounts of cis-thujopsene, widdrol and cedrol (not found in Kumotooshi oil) that appear to

be inherited as a linked group in the hybrids in a Mendelian fashion, with a second (dominant/ recessive) gene involved. PCO (Principal Coordinates analysis) using character weights of Fs (F-ratios from one-way ANOVA between the parents) was found to be the most effective method to separate the parents and their hybrids. PCA (Principal Components Analysis) and PCO using equally weighted characters were found to be ineffective in detecting hybrids, as too much weight is given to characters that do not vary among parents. In *Cryptomeria*, Adams and Tsumura (2012) found that the hybrids clustered in two groups: those with and those without the cis-thujopsene/ widdrol/ cedrol suite and that several hybrids' oils were very similar to the Haava parents' oil (Fig. 1).

Both quantitative variation and simple dominance have been reported in the inheritance of terpenes of Douglas fir (von Rudloff, 1984; von Rudloff and Rehfeldt, 1980) and Scots Pine (Pohjola, et al., 2006).

The terpene patterns of Douglas-fir showing inland and coastal groups have recently been re-confirmed (Adams et al. 2012) as the study was extended to cover the entire range of Douglas-fir to southern Mexico. Only two major chemical types were found (inland or interior and coastal).

The purposes of the present paper are to report on a complete analysis of the volatile leaf essential oil of two cultivars of *Pseudotsuga menziesii* var. *menziesii* (coastal) and var. *glauca* (inland) and their F₁ hybrids, and to compare various multivariate methods in the recognition of hybrids using terpenoid data.

MATERIALS AND METHODS

Numerous crosses have been made between Doug Fir accessions as part of the tree improvement program by the British Columbia Ministry of Forests, Lands and Natural Resource Operations (FLNRO). Some of these crosses (and hybrids) involved coastal and inland Doug Fir. Two crosses (#16 and 42) were especially suited for the study of the inheritance of terpenes. Leaves (2 branchlets, 15-20 cm long) were collected from the four parental genotypes growing as grafts in clone banks at the Cowichan Lake Research Station, Mesachie Lake, BC (48° 49'N, 124° 08'W, 160 m), while the hybrids were collected in a single-tree randomized complete block-designed progeny test at Ladysmith, BC (49° 0', 123° 31', 150 m) when the trees were 17 y old. Cross 16: coastal parent 226 (50° 03', 125° 0', 100 m) (Adams 13111, 13200) x inland parent 267 (46° 35'N, 123° 05'W, 1200 m) (Adams 13116, 13201), and F₁ hybrids - Adams 13204 (map rec. 1133), 13205 (map rec. 827), 13206 (map rec. 694), 13207 (map rec. 669), 13208 (map rec. 587), 13209 (map rec. 565), 13210 (map rec. 639), 13211 (map rec. 1047), 13212 (map rec. 285), 13213 (map rec. 314). Cross 42: coastal parent 517 (52° 49'N, 126° 58'W, 100 m) (Adams 13124/13202) x intermediate parent 521 (52° 36'N, 127° 10'W, 200 m) (Adams 13125, 13203), Adams

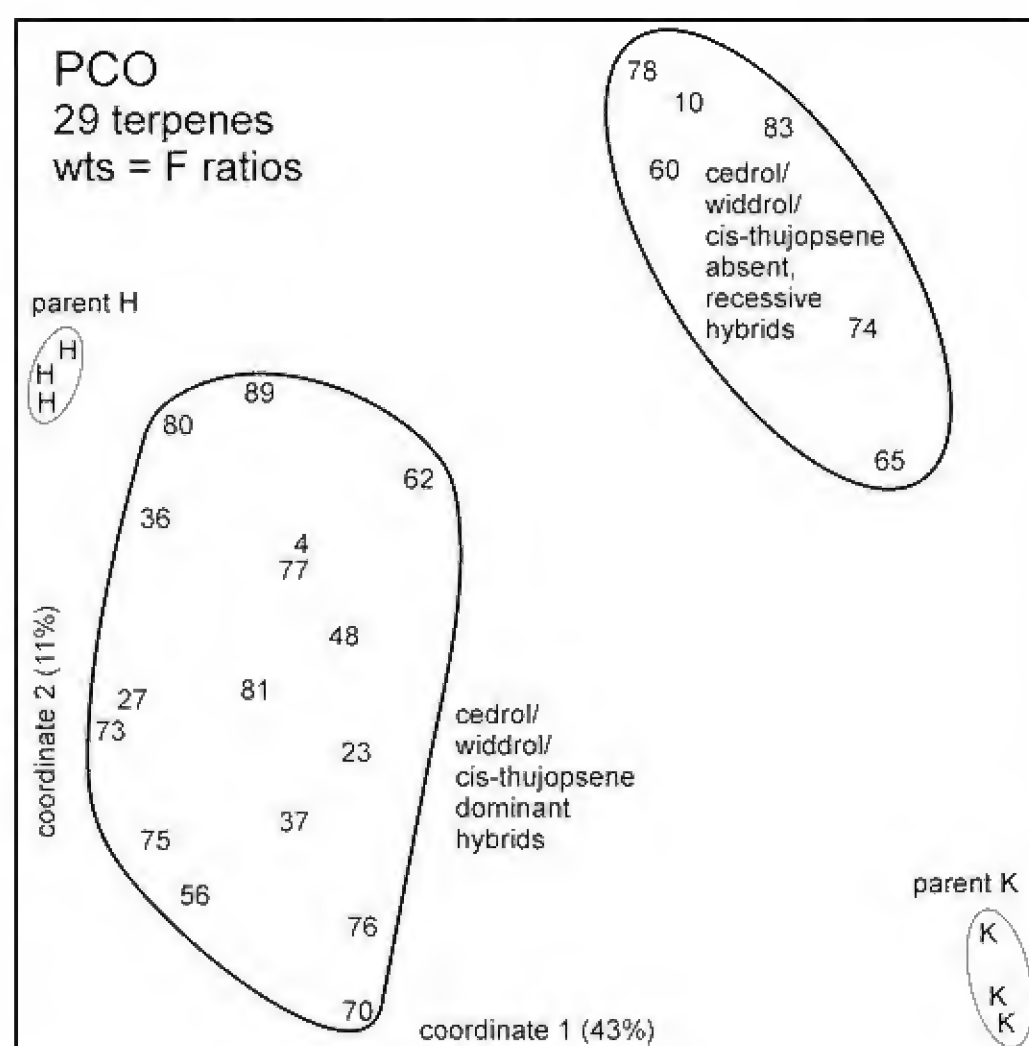


Figure 1. PCO ordination of *Cryptomeria japonica* cv. Haava . and cv. Kumotooshi, along with their 22 hybrids. Note the hybrids fall in two groups.(taken from Adams and Tsumura 2012).

13214 (map rec. 1208), Adams 13215 (map rec. 828), Adams 13216 (map rec. 490), Adams 13217 (map rec. 623), Adams 13218 (map rec. 558), Adams 13219 (map rec. 1182), Adams 13220 (map rec. 1113), Adams 13221 (map rec. 1192), Adams 13222 (map rec. 890), Adams 13223 (map rec. 287). Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Air dried (30°C, 24h) leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus and trapped in a layer of diethyl ether (Adams, 1991). The oil samples were concentrated with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using the HP Chemstation software with a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column run under the same conditions as the GCMS analysis (above).

Terpenoids (as percentage of total oil) were compared between the parents (3 replicate per parental genotype) by one-way ANOVA separately for each of the two crosses and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric similarities (Gower, 1971; Adams, 1975) were computed among all individuals using character weighting of F (where $F = MS_{\text{among parents}} / MS_{\text{within parents}}$, MS were taken from ANOVA), and equal weights (wts = 1.0). Using F for weighting factors places more importance on characters that vary more among the two parents, thus improving their separation. Principle Component Analysis (PCA) and Principal Coordinate Ordination (PCO) were performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

As reported by von Rudloff (1984) and von Rudloff and Rehfeldt (1980), coastal (var. *menziesii*) and inland (var. *glauca*) Douglas-fir differ in their concentrations of santene, camphene, β -pinene, δ -3-carene, α -terpineol, bornyl acetate, citronellyl acetate, geranyl acetate, germacrene D, α -cadinol and manool (Table 1) as well as many of the smaller components. Hybrids of cross #16, 1, 3 and 8 were generally intermediate in their composition between the parents (Table 1), whereas hybrids 2 and 9 were more like the inland parent (I267, Table 1).

Examination of variation among the hybrids reveals that only 11 terpenes were inherited as intermediate characters (Fig. 2). Several patterns are apparent (Fig. 2). In santene, tricyclene, camphene, β -pinene, 3-carene, borneol and bornyl acetate, the hybrids are generally quite intermediate between the parents (Fig. 2). However, for manool, the tendency is for the hybrids to be very much like one parent (coastal 267 in these cases), in which the compound is missing or present in trace amounts. This is suggestive of Mendelian inheritance, with absence being dominant for manool.

The balance of the 15 compounds (including percent oil yield) show transgressive variation in the hybrids (Fig. 3). Sabinene is an extreme case that was present in the hybrids in either larger or smaller amounts than in either parent (Fig. 3). Myrcene was generally smaller than in either parent. Three monoterpenes (α -terpinene, γ -terpinene and terpinolene) were generally found in hybrids in higher concentrations than either parent (Fig. 3). The hybrids had values of camphene hydrate much like inland parent 267. 4-terpineol was generally intermediate, except for one hybrid with a value of 7.5%. In contrast, α -terpineol is smaller or about the same as its inland 267 parent (Fig. 3). Citronellyl acetate (and

geranyl acetate) is similar to 4-terpineol in that the hybrids are generally intermediate with several outliers that are larger than either parent (Fig. 3). Nearly all the hybrids have only small amounts of germacrene D (and α -cadinol). As in the case of manool (above), this is suggestive of Mendelian inheritance, with absence being dominant.

The yields of oil (on a oven dry wt. basis) are of particular interest to tree breeding in Douglas fir as the amount of oil is a deterrent to deer browsing (Radwan and Ellis 1975). The loss of seedlings to deer browsing is a serious problem in reforestation with Douglas fir (and other conifers) in the Pacific northwest. So it is of interest that coastal fir (226, var. *menziesii*) is very low in oil, whereas inland (267, var. *glauca*) has twice the amount of oil (Table 1). One hybrid had the same concentration as the coastal parent, and several were intermediate (Fig. 4). However, 4 of the 10 hybrids had much larger oil yields and 2 hybrids had 4 times as much oil as their coastal parent (Fig. 4., 0.85%). Clearly, there is sufficient variation in oil yields for considerable selection for resistant Douglas-firs.

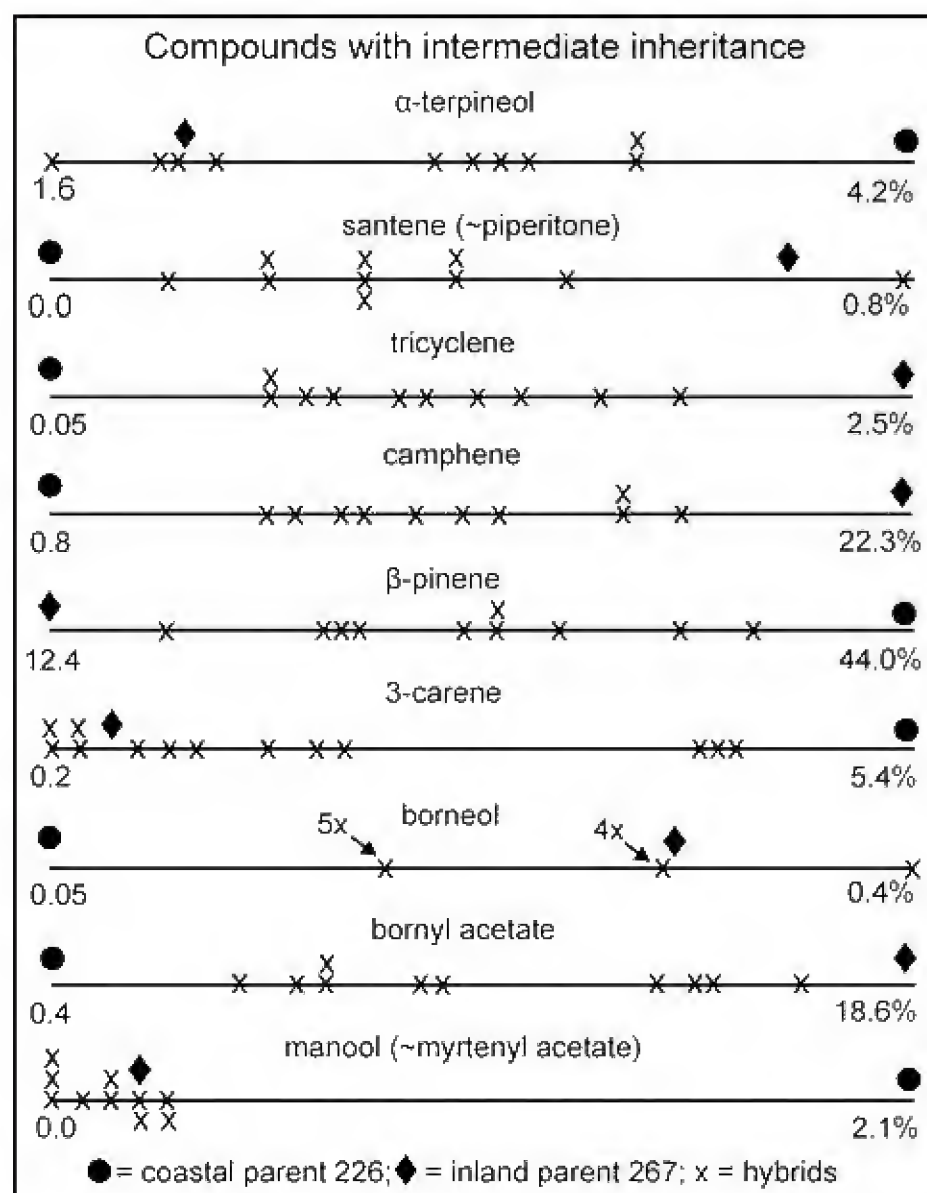


Figure 2. Compounds with intermediate inheritance in the hybrids.

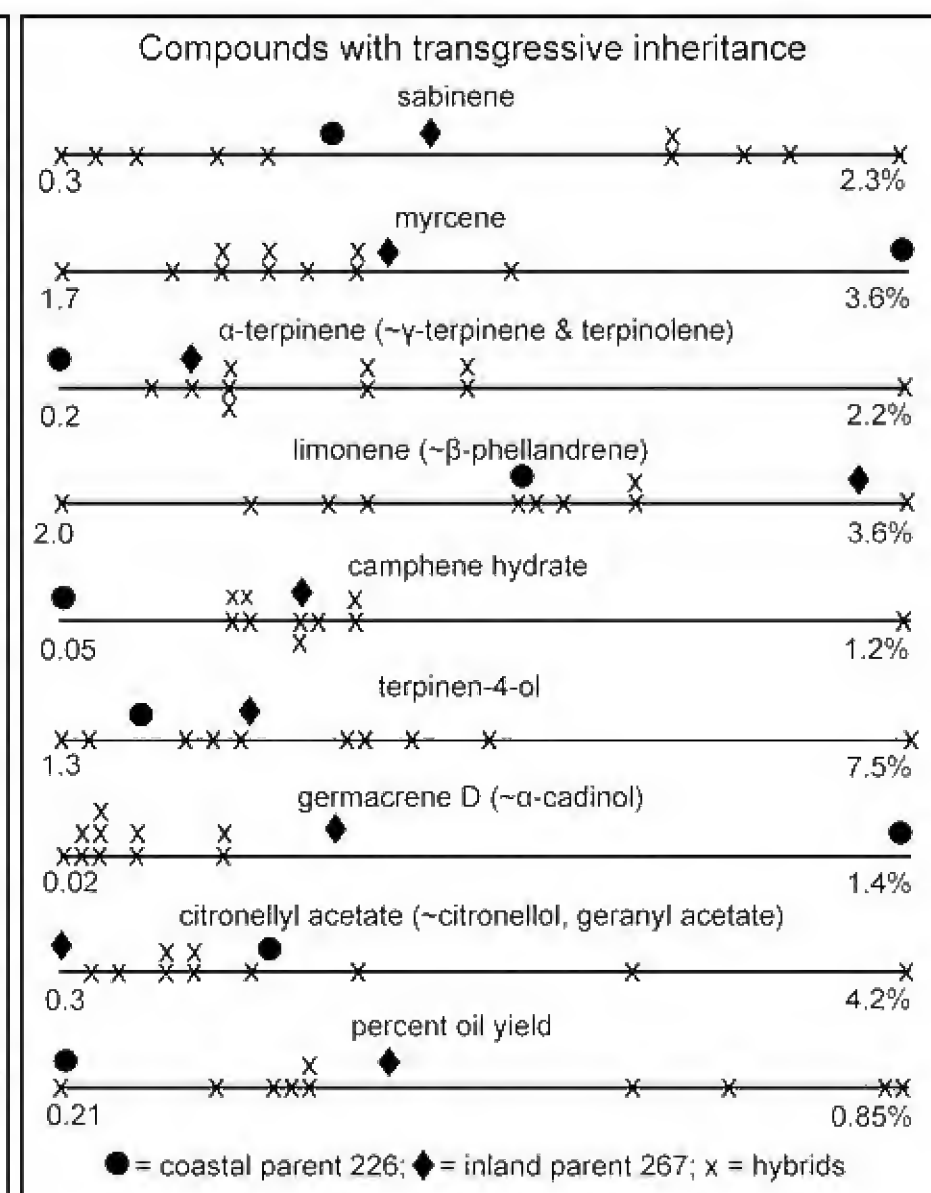


Figure 3. Compounds with transgressive inheritance in the hybrids.

PCO ordination of the parents and ten F_1 hybrids (Fig. 4) using character weights of F (from ANOVA) show the parents ordinated on axis 1 (28% of the variance). The hybrids are generally ordinated between the parents but removed on axis 2 (24%). The classical U triangle (see Adams, 1982 for detailed discussion) is not apparent (Fig. 4). Hybrids 1, 3, 5, 7, 8, 10 behave as if the components are quantitatively inherited but hybrids 2, 4, 6, 9 are more like the inland parent 267 (Fig. 4). Based on the computer simulations and morphological data from sunfish hybrids (Adams, 1982), one would conclude that figure 4 indicates a hybrid swarm with back-crossing in one direction (to parent 267). However, this is not correct, as all of the plants (1-10) are F_1 hybrids from a cross between parents 226 and 267.

The problem of transgressive characters overly influencing similarity (and thence PCO ordination) might be corrected by truncating the terpene values to the minimum or maximum value found in either parent. For example, in figure 3, several hybrids had smaller amounts of 4-terpineol than the coastal parent 226 (1.4%) and several hybrids had larger amounts than the inland parent 267 (1.7%). The value of 4-terpineol could be truncated so that the smaller values are not less than 1.4% and the larger values not more than 1.7%. A computer program was written to truncate the data values between the values of the parents. PCO was run using F weighted character matches with truncated data values for the hybrids. The resulting PCO increased the variance removed on PCO 1 axis from 28% to 36%, with no change in the variance on PCO 2 axis (24%). Ordination (Fig. 5) shows the hybrids are more tightly clustered between the parents, but several hybrids still display dominance in inheritance towards the inland parent 267 (Fig. 5). If this were field data, one would likely conclude that both F_1 hybrids (1, 3, 5, 7, 8, 10, Fig. 5) and backcrossed individuals (cf. 2, 4, 6, 9, Fig. 5) are present; which of course, is not the case. This present example in Douglas fir seems to parallel the case in *Cryptomeria japonica* (Fig. 1, above) in which the hybrids displaying dominant inheritance of cedrol/ widdrol/ cis-thujopsene appear as backcrossed individuals in the ordination.

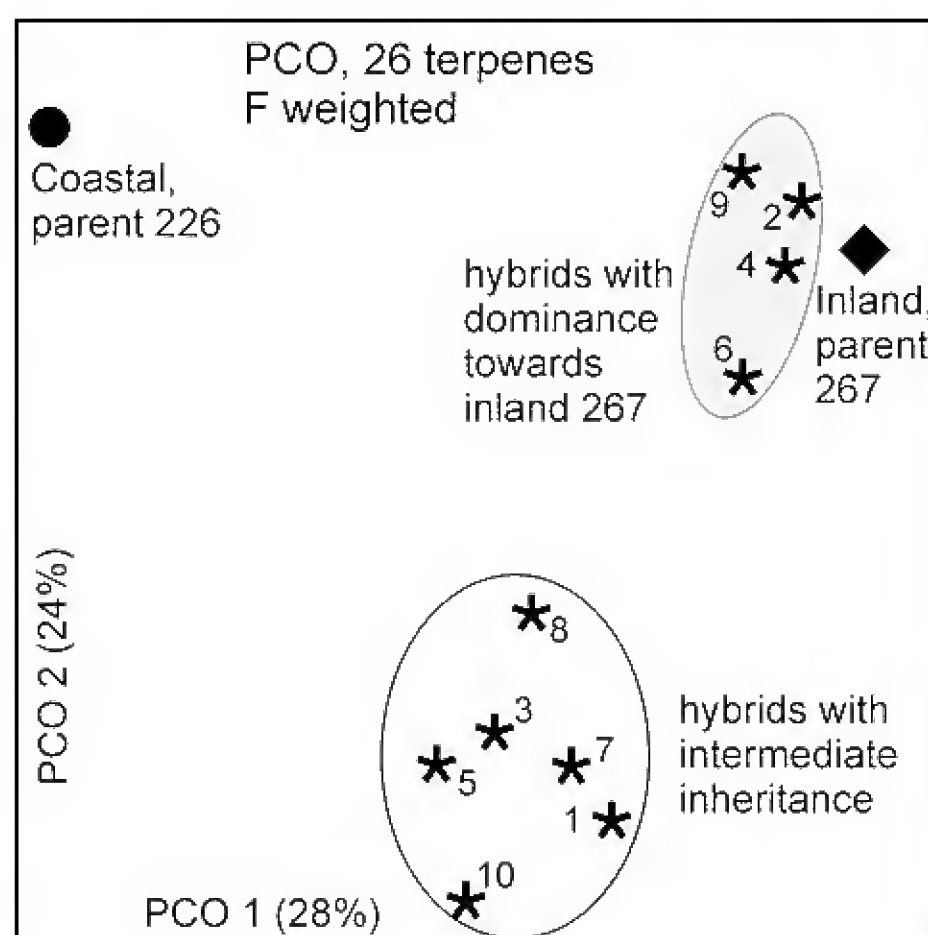


Figure 4. PCO ordination of the parents and hybrids based on 26 terpenes, F weighted.

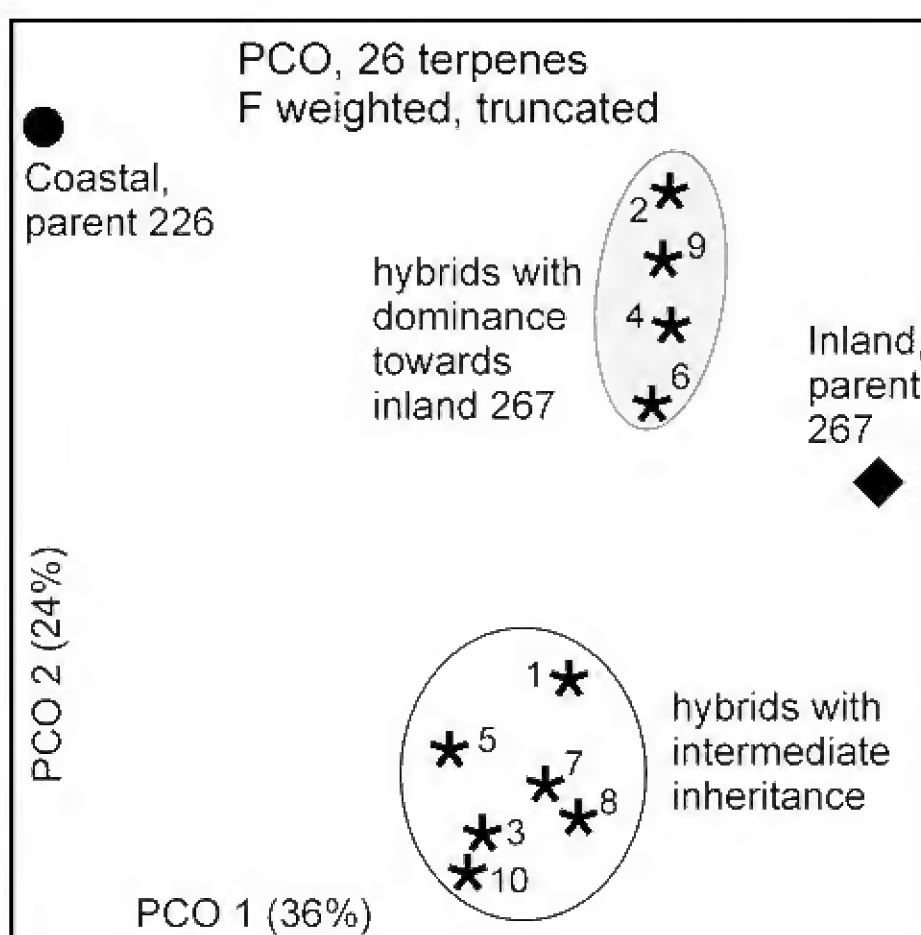


Figure 5. PCO based on 26 terpenes, F weighted, but truncated between the maximum and minimum values of the parents.

The complementation of the presence / absence of compounds is a very good indication of hybridization. However, if, by chance, most of the components that differentiate two entities have the absence state in one parent, then there may be excessive weight given to this suite of components. Examination of the patterns for the nine intermediate inheritance terpenes (Table 3), reveals the 8 of these had large weights and only α -terpineol had a lower weighting (1.81% total weights). Only two compounds showed clear dominant/ recessive inheritance, but for both of these, the hybrids were like the inland parent 267 (see Fig. 3). So using both manool and myrtenyl acetate would give extra weight to the inland type oil. For the 15 transgressive traits, the hybrids' limonene and β -phellandrene values were similar or transgressive beyond coastal 226 and each terpene had very low weights (0.12, 0.09%). Seven terpenes (Table 3, Fig. 3) displayed transgressive variation, but hybrids' values were more similar to inland parent 267. Using all 7 terpenes probably gave a bias towards inland parent 267. Camphene hydrate was selected to represent this group of 7 terpenes (Table 3). The hybrids in the third group

Table 3. Patterns of variation among coastal parent 226 and inland (interior) parent 267 and hybrids. x denotes the terpene occurrence pattern in coastal parent 226, hybrids and/ or inland parent 267. char wt = F, scaled as % total weight. char wt 1 is the original weighting based on 20 characters (Fs, scaled to % total), char wt 2 is the char weight based on 10 selected characters to balance modes between the parents (Fs, scaled to % total).

cpd	coastal parent 226	hybrids	inland parent 267	char wt 1	char wt 2
<u>intermediate (9)</u>					
α -terpineol	x	x	x	1.81	0
santene	x	x	x	7.27	10.45
piperitone	x	x	x	7.27	10.44
tricyclene	x	x	x	7.00	10.06
camphene	x	x	x	6.75	9.70
β -pinene	x	x	x	3.48	5.00
3-carene	x	x	x	8.99	12.91
borneol	x	x	x	4.55	6.53
bornyl acetate	x	x	x	6.96	10.00
<u>dominant/ recessive (2), 2 hybrids' cpds more like inland parent 267</u>					
manool		x	x	5.90	8.47
myrtenyl acetate		x	x	7.27	0
<u>transgressive (15), 7 hybrids' cpds more like inland parent 267, 2 hybrids' cpds more like coastal 226</u>					
limonene	x-transgressive	x		0.12	0
β -phellandrene	x-transgressive	x		0.09	0
citronellyl acetate	x-transgressive	x	x	4.29	6.17
citronellol	x-transgressive	x	x	7.27	0
geranyl acetate	x-transgressive	x	x	3.85	0
group total wt. 15.62%					
myrcene		x	x-transgressive	0.56	0
α -terpinene		x	x-transgressive	2.26	0
γ -terpinene		x	x-transgressive	1.10	0
terpinolene		x	x-transgressive	0.05	0
camphene hydrate		x	x-transgressive	5.54	7.96
germacrene D		x	x-transgressive	2.67	0
α -cadinol		x	x-transgressive	2.26	0
group total wt. 14.44%					
terpinen-4-ol	transgressive past both parents values			0.13	0
% oil yield	transgressive past both parents values			1.60	2.30
sabinene	transgressive past both parents values			0.94	0

(citronellyl acetate, citronellol, geranyl acetate, Table 3) tended to be more like the coastal parent 226. Citronellyl acetate was selected to represent this group. Percent oil yield was selected from the fourth group. Thus, a group of 11 terpenes and percent oil yield was selected for PCO using F wts. and truncated values. The resulting ordination (Fig. 6) shows nearly the same pattern as seen with 26 F weighted, truncated terpenes (Fig. 5). This seems surprising, but examination of the total weighting for the first transgressive group of terpenes like with hybrids like the coastal parent 226 (Table 3) is 15.62% vs. 14.44% for the second group (hybrids like inland parent 267). So selecting citronellyl acetate (wt. = 6.175) and camphene hydrate (wt. = 7.96%) apparently made little difference in the ordination (Fig. 6).

This result is both discouraging and encouraging. Even with the data distributions for both parents and hybrids, selecting a 'better' reduced set of 12 terpenes had little effect. On the other hand, merely running ANOVA between the parents and truncating the transgressive terpenes resulted in a good ordination that is similar to the '12 best terpenes'. In cases involving putative natural hybridization, one may not be able to 'select better terpenes', so the more general approach is promising.

A second set of data was available from crosses between coastal parent, 517 (var. *menziesii*) and an intermediate parent, 521 (possibly a natural hybrid between var. *glauca* and var. *menziesii*). The oils from these parents are shown in table 2 along with some hybrids. Hybrids 1, 5 and 7 are generally intermediate in their composition between the parents (Table 2), whereas the terpenes of hybrids 9 and 10 are more like the coastal parent 517 (C517, Table 2).

Examination of the variation among the hybrids reveals that only 2 terpenes are inherited as intermediate characters (Fig. 7). β -pinene shows the most intermediate pattern of any compound with nearly all values ranging between the parents (Fig. 7). The unknown sesquiterpene alcohol (KI 1627) also presented intermediate values, although several were very similar (near zero) as found in parent 512 (Fig. 7).

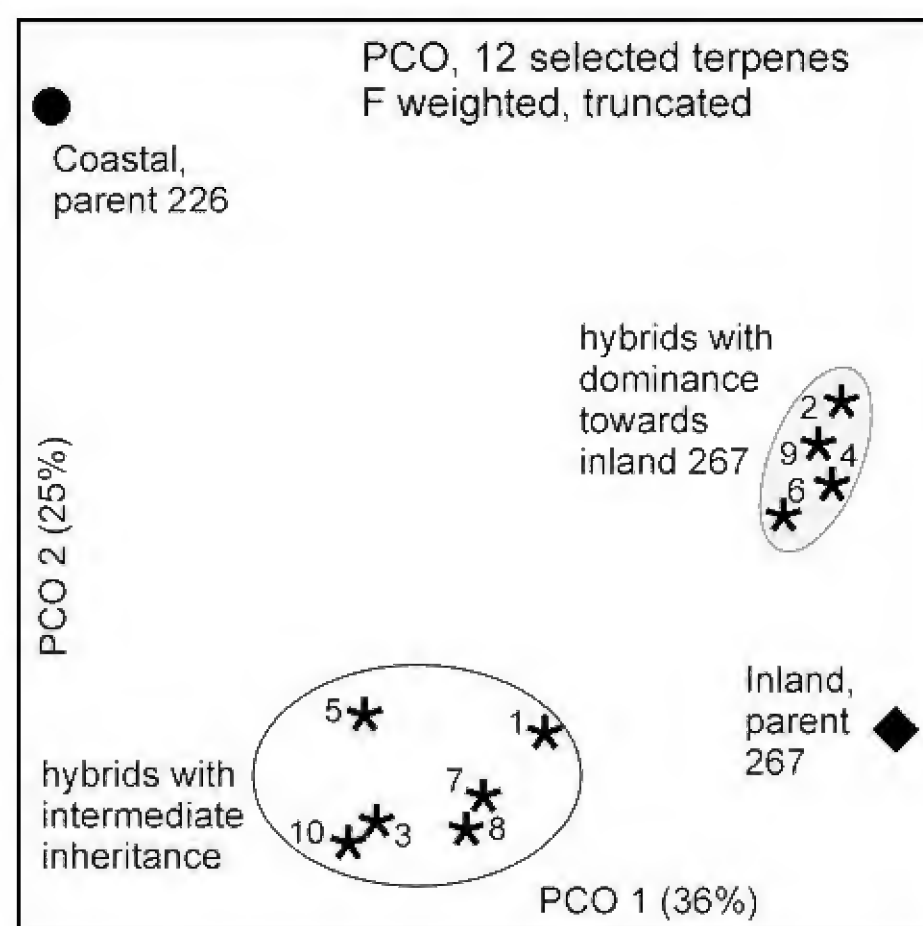


Figure 6. PCO using 12 selected terpenes.

Eight compounds displayed the appearance of dominance/ recessive inheritance: camphene, germacrene D, viridiflorene, bornyl acetate, cembrene, thunbergol, manool and abienol (Fig. 7). Camphene is somewhat intermediate (Fig. 7), with only small amounts like parent 521. Two of the 8 had zero or very small concentrations as found in inland plant 521. Six hybrids had zero or very small amounts as found in coastal parent 517.

Percent oil yield and 9 terpenoids had transgressive variation (Fig. 8). Percent oil yield is interesting as both parents had very low oil yields (0.16, 0.23%, Table 2), yet the hybrids ranged from 0.31 to 1.04% with most yields exceeding either parent (Fig. 8). Again, breeding for increased oil yields for herbivore browsing resistance seems favorable between these parents. Additional research is ongoing on in this field of chemo-resistance (Burney and Jacobs, 2012; Kimball et al. 2011). In several cases most of the hybrids either exceeded the parents or were less than either parent (see Fig. 8: α -pinene, 3-carene, terpinolene, 4-terpineol, bornyl acetate, and manool).

PCO using F (from ANOVA) weighted characters removed 37 and 22% percent of the variation among the parents and hybrids on the first two coordinates. Ordination reveals the hybrids are in two groups (Fig. 9), with one group (1, 4, 5, 6, 7, 8) more intermediate between the parents (as expected for hybrids). The second group (2, 3, 9, 10) are still intermediate, but closer to coastal parent 517 (Fig. 9).

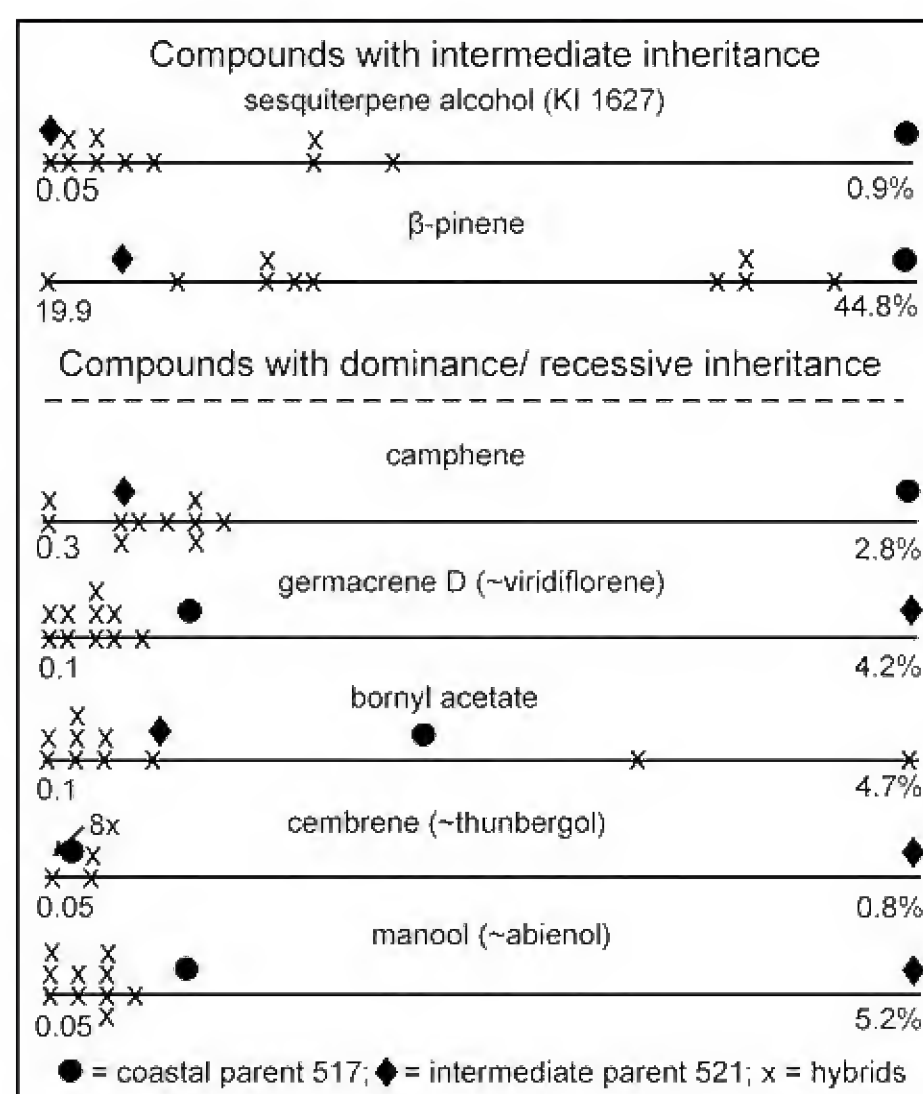


Figure 7. Compounds with intermediate or showing dominance towards one of the parents.

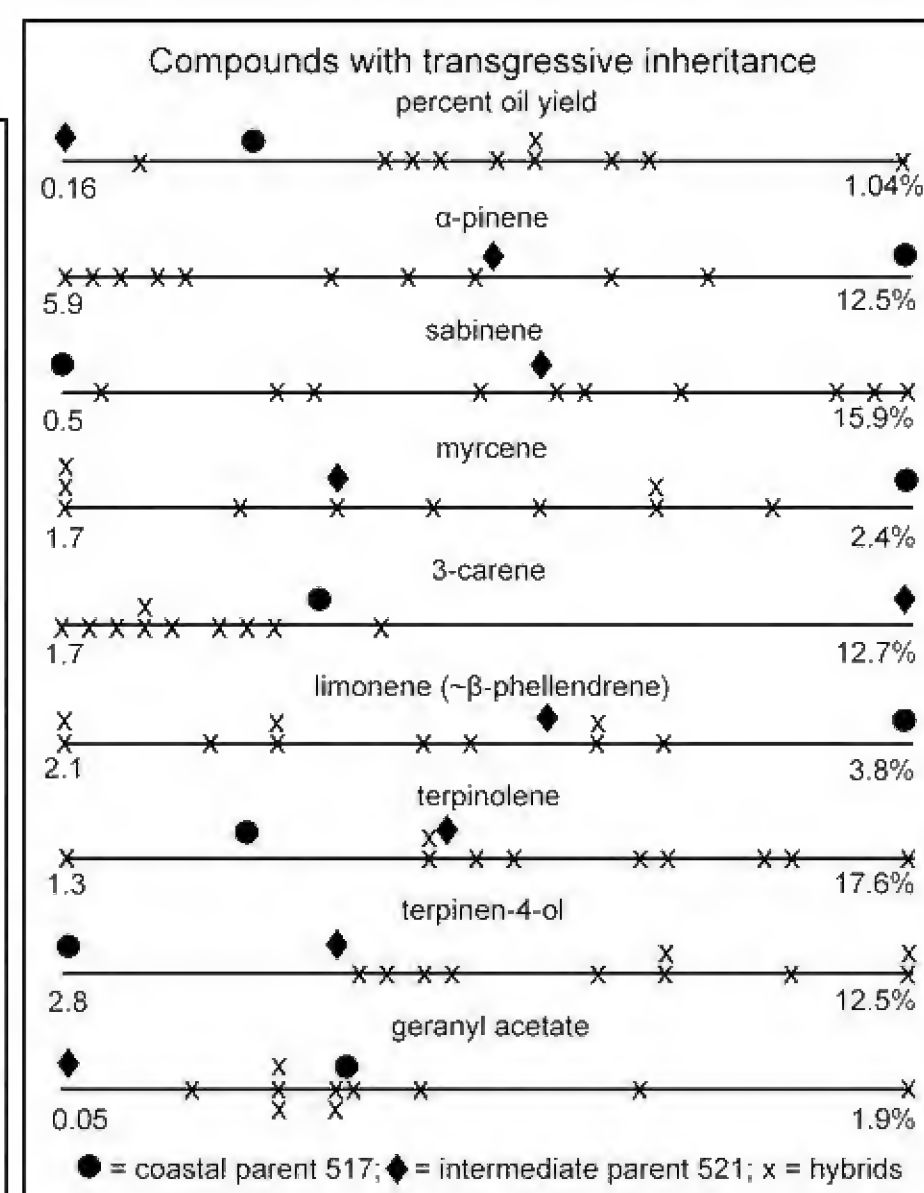


Figure 8. Percent oil yield and 9 terpenoids that had transgressive values in the hybrids.

A second PCO with F weighting and truncated data values removed 46 and 23% of the variance. There is an improvement in the percent variance accounted for in PCO using truncated data, just as with the previous data set (cf. Figs. 4, 5). Ordination shows (Fig. 10) the clusters are a little tighter and the two hybrid groups are now essentially connected into one group. In both the previous cross (coastal 226 x inland 267) and this cross (coastal 517 x intermediate 521), the use of truncated data values appears to improve the detection of hybrids.

Examination of the patterns for the eight dominant/ recessive inheritance terpenes (Table 4), shows only 2 terpenes (camphene, bornyl acetate) represent the pattern of intermediate parent 521 and 6 typify the pattern of coastal parent 517. Thus, if all 8 are used, there is a bias towards terpenes representative of the coastal parent 517. To correct this bias, one could select 2 from each group (camphene, bornyl for parent 521 type), (germacrene D, manool for coastal parent 517 type). Among the transgressive characters, 2 characters were chosen to represent the coastal parent 517 type (percent oil yield, 3-carene) and 2 were chosen to represent the parent 521 type (terpinolene, terpinen-4-ol). Including the 2 terpenes showing intermediate inheritance (β-pinene, KI1627), this gives 10 characters (Table 4) of which 4 are associated with the coastal parent 517 and 4 associated with the parent 521.

PCO using these 10 characters, F weighted, and truncated shows a more intermediate pattern (Fig. 11). Note that the variance removed is very similar to previous ordinations (Figs. 9, 10), yet the placement of the hybrids is more intermediate and in a tighter group. Although this approach seems to be very useful in the present case of known parents and hybrids, it may be difficult to apply to natural field hybridization cases. It is interesting that hybrids 3, 8, 9, 10 are quite separated with F wts (Fig. 9), then

less separated when the transgressive data values are truncated to values of the parents (Fig. 10), and finally using 10 selected terpenes, F weighted, truncated, the hybrids 3, 8, 9, 10 are completely intermingled with hybrids 1, 2, 4, 5, 6, 7 (Fig. 11). Clearly, in this case, selecting representative terpenes from both parents to balance the modes of inheritance benefited the ordination by placing the hybrids in a more intermediate position. This allows the investigator to correctly assign these as hybrids. In addition, this provides for the possibility that in a natural field hybridization case, back-crossed individuals may be ordinated between the hybrids and the back-crossed parent.

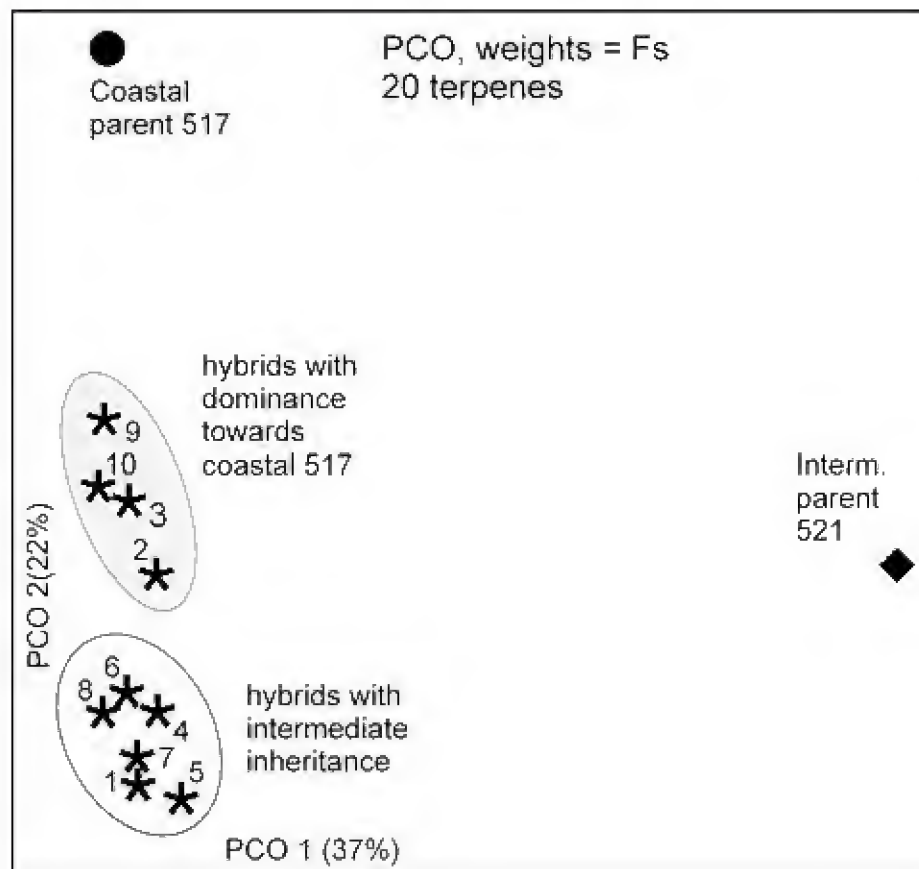


Figure 9. PCO with F weighted, 20 terpenes.

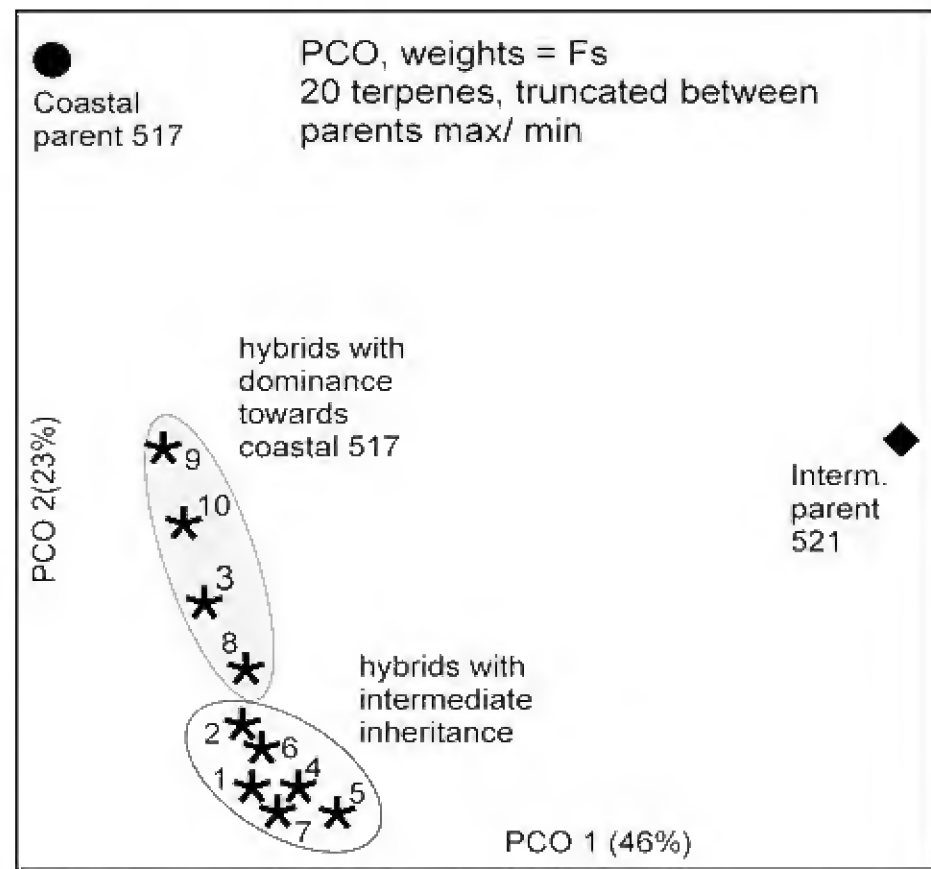


Figure 10. PCO with F weighted, 20 terpenes, truncated between parents.

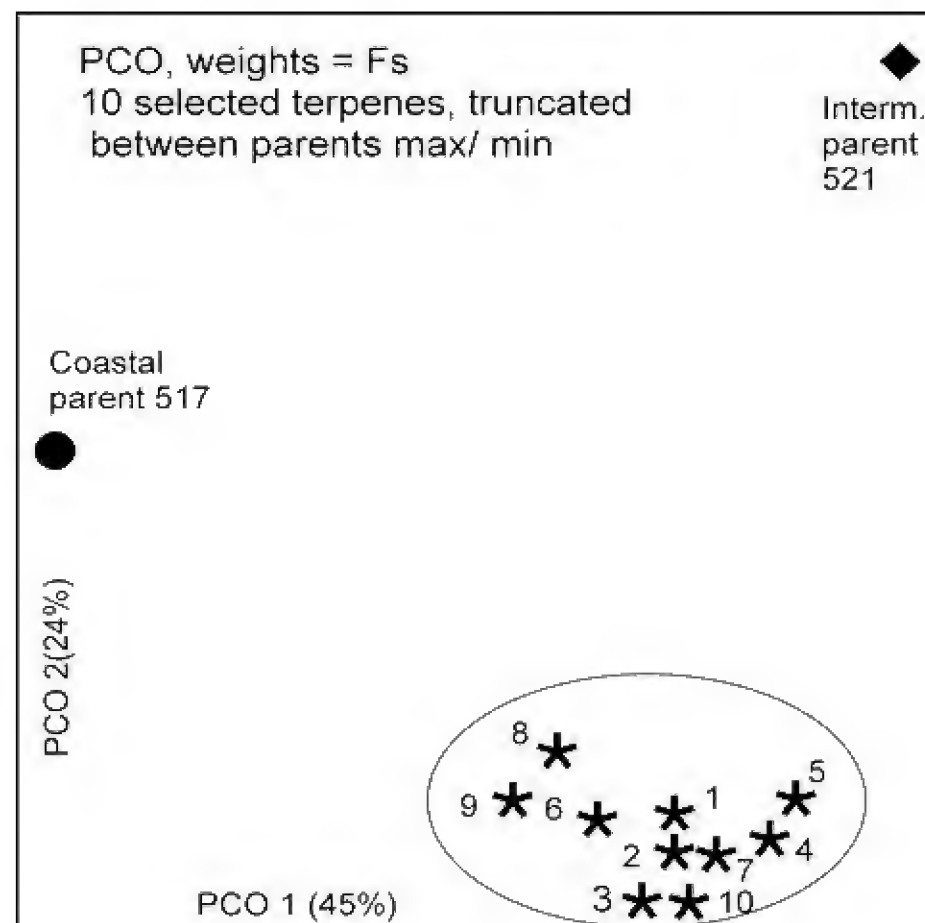


Figure 11. PCO using 10 selected characters to balance representation of modes equally between the parents. Notice the hybrids are in a tighter group and very intermediate between the parents.

Table 4. Patterns of variation among parents 517 and 521 and hybrids. x denotes the terpene occurrence pattern in coastal parent 517, hybrids and/ or parent 521. char wt = F, scaled as % total weight. char wt 1 is the original weighting based on 20 characters (Fs, scaled to % total), char wt 2 is the char weight based on 10 selected characters to balance modes between the parents (Fs, scaled to % total).

cpd	coastal parent 517	hybrids	intermediate parent 521	char wt 1	char wt 2
intermediate (2)					
KI 1627	x	x	x	9.41	20.44
β-pinene	x	x	x	2.44	5.31
dominant/ recessive (8), 2 hybrids like 521, 6 hybrids like 517					
camphene		x	x	7.69	16.70
bornyl acetate		x	x	5.46	11.86
germacrene D	x	x		7.69	16.70
viridiflorene	x	x		8.31	0
cembrene	x	x		9.23	0
thunbergol	x	x		9.93	0
manool	x	x		5.65	12.28
abienol	x	x		6.91	0
transgressive (10), 7 hybrids' cpds more like 521, 3 hybrids' cpds more like 517					
% oil yield	x-transgressive	x		0.65	1.42
3-carene	x-transgressive	x		2.79	6.07
geranyl acetate	x-transgressive	x		8.73	0
				group total wt. 12.17%	
terpinolene		x	x-transgressive	2.02	4.37
terpinen-4-ol		x	x-transgressive	2.23	4.83
α-pinene		x	x-transgressive	0.48	0
sabinene		x	x-transgressive	9.59	0
myrcene		x	x-transgressive	0.28	0
limonene		x	x-transgressive	0.22	0
β-phellandrene		x	x-transgressive	0.27	0
				group total wt. 15.09%	

CONCLUSION

From the previous study of *Cryptomeria japonica* synthetic hybrids (Adams and Tsumura, 2012) and the present study of two crosses of *Pseudotsuga menziesii*, it is clear that the detection of hybridization may prove to be difficult due to the presence of linkage groups (*Cryptomeria japonica*) and/ or the presence of terpenes are inherited as dominant/ recessive traits in which several compounds co-vary in the direction of one of the parents (present study). This study may illustrate inheritance differences between a wide genetic cross (226, var. *menziesii*, x 267, var. *glauca*) and a more narrow cross (517, var. *menziesii*, x 521, intermediate between var. *menziesii* and var. *glauca*, possibly a hybrid). Notice that comparing table 3 (wide cross) and table 4 (narrow cross):

	wide genetic cross	narrow genetic cross
no. intermediate cpds.	9	2
no. dominant/ recessive cpds.	2	8
no. transgressive cpds.	15	10

One might expect more transgressive cpds in a wide genetic cross as there should be more incompatible pathways activated in a wider cross. Thus, the taxonomically wider the cross, the higher the probability that some independently evolved pathways exist in the parental lines. And as a consequence, the hybrids will contain disparate gene regulators, so gene control may be disrupted, leading to over/under expression of some compounds. The presence of more intermediate compounds in the wide cross and more dominant/ recessive compounds in the narrow cross is difficult to explain.

At present, it appears that utilizing F (from ANOVA between the parents) to weight the character matches and truncating transgressive characters back to the range found in the parents, aids in producing ordinations in which the hybrids are intermediate between the parents. The use of selected components that are typical for each parent and using the same numbers of parental representatives helped in ordinating the hybrids intermediate between the parents in one of the two cases examined in this study. In a study of natural field hybridization, the terpenes should be useful in the detection of hybrids but it may be difficult to accurately classify back-crossed or F₂ individuals.

ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. Thanks also to Keith Bird and Lisa Hayton from MFLNRO for sample collections and shipment.

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Table 1. Comparison of leaf oil compositions for *Pseudotsuga menziesii* oils. var. *menziesii*: (coastal parent) C226, var. *glauca* (inland parent) I267, with several hybrids. Compounds in bold face appear to separate the parents, had significant F ratios and were used in numerical analyses.

KI	cpd	C226	I267	F ₁ #1	F ₁ #3	F ₁ #8	F ₁ #2	F ₁ #9
	% yield	0.21	0.42	0.74	0.37	0.43	0.49	0.40
884	santene	-	0.7	0.3	0.2	0.3	0.8	0.5
921	tricyclene	t	2.5	1.4	0.9	1.0	1.9	1.7
924	α -thujene	t	t	0.2	0.1	0.2	0.3	0.1
932	α -pinene	16.0	15.1	13.4	14.2	15.1	12.3	13.5
946	camphene	0.8	22.3	12.0	9.0	9.7	16.9	16.3
969	sabinene	0.7	1.2	1.8	0.8	0.5	2.3	0.3
974	β-pinene	44.1	12.4	24.2	39.1	36.0	17.1	29.4
988	myrcene	3.6	2.4	2.1	2.4	2.7	1.7	2.1
1002	α -phellandrene	-	0.1	0.2	0.1	0.1	0.1	0.1
1008	δ-3-carene	5.4	0.4	0.5	0.7	2.0	2.1	1.2
1014	α-terpinene	0.2	0.5	1.2	0.6	0.6	1.0	0.4
1020	p-cymene	0.4	0.3	0.3	0.5	0.5	0.8	0.4
1024	limonene	2.9	3.5	2.5	2.9	3.1	2.9	3.6
1025	β-phellandrene	3.0	3.5	2.5	2.9	3.1	2.9	3.7
1032	(Z)- β -ocimene	-	0.3	t	t	t	t	t
1054	γ-terpinene	0.5	0.9	2.1	1.0	1.0	1.7	0.7
1065	cis-sabinene hydrate	-	t	t	t	t	t	t
1086	terpinolene	2.4	2.7	5.4	3.3	3.2	5.5	2.6
1089	6-camphenone, isomer	-	t	t	t	t	t	t
1095	linalool	0.2	0.3	0.3	0.3	0.3	t	t
1118	cis-p-menth-2-en-1-ol	t	t	0.2	t	0.1	0.2	t
1118	endo-fenchol	0.2	-	t	0.1	0.2	t	t
1135	trans-pinocarveol	0.3	-	t	-	t	t	t
1136	trans-p-menth-2-en-1-ol	-	t	t	t	t	t	t
1141	camphor	t	t	t	t	t	t	t
1145	camphene hydrate	t	0.4	0.5	0.3	0.3	0.5	0.4
1165	borneol	t	0.3	0.2	0.2	0.3	0.3	0.3
1174	terpinen-4-ol	1.4	1.7	4.3	2.6	2.2	3.7	1.3
1179	p-cymen-8-ol	t	-	t	t	t	t	t
1186	α-terpineol	4.2	1.9	3.3	3.0	3.4	2.0	2.2
1195	myrtenal	0.4	-	t	t	t	t	t
1196	ethyl octanoate	-	0.1	t	0.3	0.1	t	t
1223	citronellol	0.4	-	1.4	0.8	0.6	0.7	0.4
1232	thymol, methyl ether	-	0.2	t	-	-	t	t
1249	piperitone	-	0.8	0.5	0.7	0.8	t	t
1287	bornyl acetate	0.4	18.6	9.1	7.4	7.5	14.5	13.3
1298	trans-pinocarvyl acetate	0.2	t	t	t	t	t	t
1323	methyl decanoate	-	0.1	t	t	t	t	t
1324	myrtenyl acetate	0.1	-	t	t	t	t	t
1350	citronellyl acetate	1.4	0.3	3.0	0.9	0.5	1.8	0.7
1357	(2E)-undecenal	0.1	-	t	t	t	t	t
1379	geranyl acetate	0.4	0.1	1.6	0.5	0.2	0.8	0.2
1395	ethyl decanoate	-	t	t	t	t	t	t
1452	α -humulene	0.2	0.2	t	t	t	t	t
1480	germacrene D	1.4	0.5	t	t	t	0.2	0.2
1522	δ -cadinene	0.2	0.1	t	t	t	t	t
1608	humulene epoxide II	0.1	t	t	t	t	t	t
1616	43,81,161,222	0.7	0.5	0.5	0.5	0.5	0.6	0.5

1627	43,119,159,220	0.8	0.8	-	0.4	0.4	0.7	0.6
1638	epi- α -cadinol	0.2	0.4	-	t	t	0.1	t
1638	epi- α -muurolol	0.2	0.4	-	t	t	0.1	t
1644	α -muurolol	t	t	-	t	t	t	t
1652	α-cadinol	1.0	0.4	t	0.2	0.1	0.4	0.4
1814	hexadecanal	t	0.1	0.6	0.7	0.5	0.3	t
1937	cembrene	0.1	0.2	t	t	t	t	t
1943	iso-cembrene	t	0.1	t	t	t	t	t
1987	manoyl oxide	0.2	t	t	t	t	t	t
2048	thunbergol	0.1	0.2	t	t	t	t	t
2056	manool	2.1	0.2	t	0.2	0.3	t	0.1
2300	tricosane(C23)	0.5	0.2	0.2	0.2	0.1	0.1	0.3

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Table 2. Comparison of leaf oil compositions for *Pseudotsuga menziesii* oils. var. *menziesii*: coastal parent - C517, intermediate - I521, and several hybrids. Compounds in bold face appear to separate the parents and were used in numerical analyses.

KI	cpd	C517	I521	F ₁ #1	F ₁ #5	F ₁ #7	F ₁ #9	F ₁ #10
	% yield	0.23	0.16	1.04	0.64	0.73	0.31	0.49
884	santene	t	t	t	-	-	t	t
921	tricyclene	0.3	t	t	t	t	t	t
924	α -thujene	0.1	0.2	0.5	0.7	0.5	0.3	0.4
932	α-pinene	12.5	9.2	6.9	6.1	6.7	8.9	8.5
946	camphene	2.8	0.4	0.4	0.3	0.4	0.6	0.6
969	sabinene	0.5	9.8	11.8	15.2	9.6	3.5	1.2
974	β-pinene	44.8	21.1	26.7	19.9	25.3	39.2	43.0
988	myrcene	2.4	1.9	1.7	1.7	1.8	2.0	2.1
1002	α -phellandrene	0.1	0.2	0.2	0.4	0.4	0.2	0.3
1008	δ-3-carene	5.6	12.7	9.5	2.5	1.7	3.6	3.5
1014	α -terpinene	0.8	0.7	2.5	4.1	3.5	1.6	1.9
1020	p-cymene	0.2	0.4	0.5	0.9	0.9	1.3	0.9
1024	limonene	3.8	3.1	2.1	2.3	2.5	2.9	2.8
1025	β-phellandrene	3.9	3.1	2.1	2.3	2.5	2.9	2.8
1032	(Z)- β -ocimene	t	t	0.1	t	t	t	t
1044	(E)- β -ocimene	t	0.3	0.1	t	0.4	t	0.6
1054	γ -terpinene	1.3	1.5	4.2	6.5	5.4	2.7	3.2
1065	cis-sabinene hydrate	t	t	0.4	0.3	0.2	0.2	0.2
1086	terpinolene	4.3	8.4	11.2	17.6	13.6	8.0	10.2
1089	6-camphenone, isomer	t	t	t	t	t	t	t
1095	linalool	0.2	0.5	0.5	0.3	0.3	0.3	0.3
1118	cis-p-menth-2-en-1-ol	0.1	t	0.6	0.6	0.6	0.4	0.4
1118	endo-fenchol	t	t	t	t	t	0.2	t
1135	trans-pinocarveol	t	t	0.4	0.5	0.4	0.3	0.3
1136	trans-p-menth-2-en-1-ol	t	t	t	t	t	t	t
1141	camphor	t	t	t	t	t	t	t
1145	camphene hydrate	0.2	t	0.2	t	0.1	0.2	0.1
1165	borneol	t	t	t	t	t	t	t
1172	cis-pinocamphone	t	0.4	t	t	t	t	t
1174	terpinen-4-ol	2.8	5.7	9.4	12.4	12.4	6.7	7.1
1179	p-cymen-8-ol	t	0.2	t	0.2	0.2	0.2	0.2
1186	α -terpineol	4.4	3.5	2.3	2.0	3.3	3.6	2.7
1195	myrtenal	t	t	0.2	t	t	t	t
1196	ethyl octanoate	t	t	0.2	0.2	0.2	0.2	0.1
1223	citronellol	t	t	2.1	0.2	1.1	0.3	0.8
1232	thymol, methyl ether	t	t	t	t	t	t	t
1249	piperitone	t	t	0.1	t	t	0.2	t
1287	bornyl acetate	2.3	0.6	0.1	0.1	0.2	4.7	0.3
1298	trans-pinocarvyl acetate	t	t	t	t	t	t	t
1323	methyl decanoate	t	t	t	t	t	t	t
1324	myrtenyl acetate	t	t	t	t	t	t	t
1350	citronellyl acetate	0.5	0.3	3.1	0.4	1.3	0.7	1.1
1357	(2E)-undecenal	t	t	t	t	t	t	t
1379	geranyl acetate	0.6	t	1.9	0.3	1.3	0.5	0.8
1395	ethyl decanoate	t	t	t	t	t	t	t
1452	α -humulene	0.1	t	t	t	t	t	t
1480	germacrene D	0.6	4.2	0.1	0.2	0.1	0.1	0.2
1496	viridiflorene	t	0.5	0.1	t	0.1	0.1	t

1508	germacrene A	t	0.4	t	t	t	t	t
1522	δ -cadinene	0.2	t	t	t	t	0.1	t
1608	humulene epoxide II	t	t	t	t	t	t	t
1616	43,81,161,222	t	-	t	t	t	t	t
1627	43,119,159,220	0.9	-	0.1	t	0.1	0.3	0.1
1638	epi- α -cadinol	0.1	t	t	t	0.1	t	t
1638	epi- α -muurolol	0.2	t	t	t	t	t	t
1644	α -muurolol	t	t	t	t	t	t	t
1652	α -cadinol	0.4	0.3	0.1	t	t	0.3	0.4
1814	hexadecanal	t	t	t	t	t	0.1	t
1937	cembrene	t	0.8	t	t	t	t	t
1943	iso-cembrene	t	0.3	t	t	t	t	t
1987	manoyl oxide	0.3	0.3	t	t	t	t	t
2048	thunbergol	t	1.5	t	t	t	t	t
2056	manool	1.3	5.2	0.4	0.1	0.3	0.2	0.3
2149	abienol	0.2	1.1	t	t	t	t	t
2300	tricosane(C23)	0.1	0.3	t	t	t	t	t

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Hybridization between *Juniperus grandis*, *J. occidentalis* and *J. osteosperma* in northwest Nevada I: Terpenes, Leviathan Mine, Nevada

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ABSTRACT

The volatile leaf oils of *J. grandis*, *J. occidentalis*, *J. osteosperma* and putative hybrids from near Leviathan Mine, NV were analyzed. No evidence of hybridization involving *J. occidentalis* was found. There appears to be hybridization between *J. grandis* and *J. osteosperma*. Only one tree, morphologically typical of *J. grandis*, was found in the Leviathan mine population. One shrub appeared to be, morphologically, pure *J. osteosperma*. PCO, using 49 terpenes, with character matches weighted by F (from ANOVA between parental species) produced no evidence that *J. occidentalis* was involved in hybridization with *J. osteosperma* in this population. PCO analysis (with 42 terpenes), revealed hybrids between *J. grandis* and *J. osteosperma*, and possible backcrosses to *J. osteosperma*. Analyses of 32 of the largest terpene components revealed 6 intermediates, 8 dominant/ recessives; 18 terpenes were transgressive, beyond the range of *J. grandis* or *J. osteosperma*. These transgressive components were truncated to values in the range of the putative parental species and a new PCO indicated the plants to be more intermediate. The terpene analysis seems in agreement with the haplotype data of Terry (2010). Published on-line: www.phytologia.org *Phytologia* 95(1): 58-69 (Feb. 1, 2013).

KEY WORDS: *J. osteosperma*, *J. grandis*, *J. occidentalis*, hybridization, Cupressaceae, terpenes, Leviathan mine, Nevada.

Hybridization among species of *Juniperus* in north-western Nevada was first reported by Vasek (1966) and confirmed by Terry et al. (2000) and Terry (2010). Terry et al. (2000) found cpDNA (trnL-trnF, trnS-trnG) haplotypes of *J. occidentalis* in Nevada populations of *J. osteosperma*, with lower frequencies occurring in Utah, Colorado, and Wyoming. Subsequently, Terry (2010) analyzed trnL-trnF and trnS-trnG (cpDNA) haplotypes and reported similar results (Fig. 1). Notice, all 15 trees of *J. occidentalis* in Oregon have the same haplotype and that this haplotype is also present in northwest Nevada. The Leviathan mine population was one of the most diverse populations and contained 5 haplotypes (Fig. 1).

Recently, Adams (2012a) analyzed geographic variation in the leaf essential oils of *J. osteosperma* (Torr.) Little and reported differences among the populations. However, the putative hybrid populations of northwest Nevada were not included in that study.

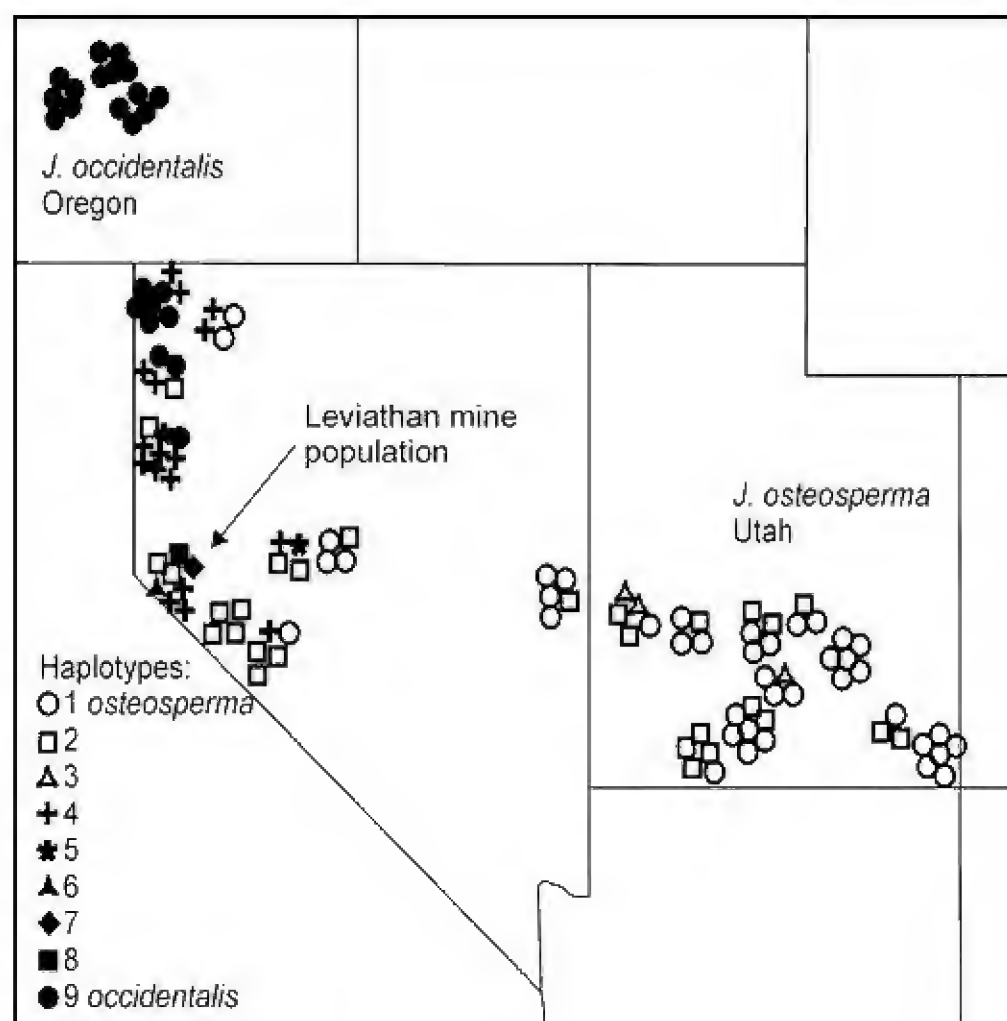


Figure 1. Distribution of haplotypes (trnL-trnF and trnS-trnG) in *J. occidentalis* and *J. osteosperma* (based on Terry, 2010).

These three western junipers occupy generally allopatric ranges (Fig. 2), with *J. grandis* favoring granitic outcrops in the high Sierra, *J. occidentalis* growing on lava beds at lower elevations in northern California and Oregon, and *J. osteosperma*, preferring the intermediate elevations in the Basin and Range region of Nevada, Utah and adjacent states; a fourth species, *J. californica*, grows in the Mojave desert foothills of southern California, thence northward in the central valley foothills (Adams 2011). Adams (2012b) found that *Juniperus grandis* and *J. occidentalis* appear to hybridize in the Beckwourth, CA area (Fig. 2) but, otherwise no evidence of gene flow between these species was found.

The Leviathan mine population, sampled by Terry (2010, popn. 16) appears to be an area of sympatry between *J. grandis* and *J. osteosperma* and subject to ancestral as well as possible current hybridization. Analysis of plants from the Leviathan mine population is the focus of this paper.

MATERIALS AND METHODS

Plant material: *J. grandis*, Adams 11963-11967, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; Adams 11968-11972, 16 km w of Sonora Jct., on CA. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA, *J. osteosperma*, Adams 1689-1699, 1701-1705, on US 6, Thistle, 40° 00' 6.9" N, 111° 29' 4.6" W, 1650 m, Utah Co., UT, Adams 12067-12071, 4 km n of Sedona, AZ, at Grasshopper Point, on Alt US 89, 34.888° N, 111.733° W, 1380m, Coconino Co., AZ, Adams 10272-10276, on NV157, Charleston Mtns., 36° 16.246' N, 115° 32.604' W, 1795 m, Clark Co., NV; Adams 11122-11124, Hancock Summit, mile 38 on US 375, 37° 26.404' N, 115° 22.703' W, 1675 m, Lincoln Co. NV; Adams 11125-11127, McKinney

Tanks Summit on US 6, 38° 07.005' N, 116° 54.103' W, 1933 m, Nye Co., NV; Adams 11134-36, 8 km s of Bridgeport, on US395, 38° 12.639' N, 119° 13.846' W, 2004 m, Mono Co., CA; Adams 11141-11143, 13 km w of Elko, on I 80, 40° 45.598' N, 115° 55.942' W, 1535 m, Elko Co., NV; Adams 11144-11146, 8 km e of Wells, on I 80, 41° 06.533' N, 114° 51.441' W, 1876 m, Elko Co., NV; Adams 11960-11962, 56 km n of Reno, NV; on US 395, 39° 54.458' N, 120° 00.322' W, 1383 m, Lassen Co., CA; Adams 11973-11977, 10 km n of CA 168 on White Mtn. Rd., 37° 20.143' N, 118° 11.346' W, 2607 m, Inyo Co., CA; Adams 11978-11982, Mahogany Flats Campground, Panamint Mtns., 36° 13.783' N, 117° 04.102' W, 2477 m, Inyo Co., CA, Adams 12323-12327, Basin, San Bernardino Mtns., 34° 16.910' N, 116° 45.306' W, 1820 m, San Bernardino Co., CA, Adams 12210-12214, ca. 1 km e of CA 18, ca. 16 km s of jct CA 18 & CA 247, n slope San Bernardino Mtns., 34° 21.213' N, 116° 50.607' W, 1393 m, San Bernardino Co., CA, Adams 12215-12219, on I15, at Bailey Rd., 35° 27.938' N, 115° 31.709' W, 1431 m, San Bernardino Co., CA. *J. occidentalis*, Adams 11940-11942, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392' N, 120° 41.207' W, 170 m, Klickitat Co.; WA, Adams 11943-11945, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44° 53.676' N, 120° 56.131' W, 951 m, Wasco Co., OR; Adams 11946-11948, 3 km sw of Bend, OR; on OR 372, 44° 02.390' N, 121° 20.054' W, 1132 m, Deschutes Co., OR; Adams 11949-11951, 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922' N, 120° 59.187' W, 1274 m, Deschutes Co., OR; Adams 11952-11954, 14 km e of Jct. OR66 & I 5, on OR66, 42° 08.044' N, 122° 34.130' W, 701 m, Jackson Co., OR; Adams 11957-11959, on CA 299, 10 km e of McArthur, CA, 41° 05.313' N, 121° 18.921' W, 1091 m, Lassen Co., CA; Adams 11995-11998 (Kauffmann A1-A3,

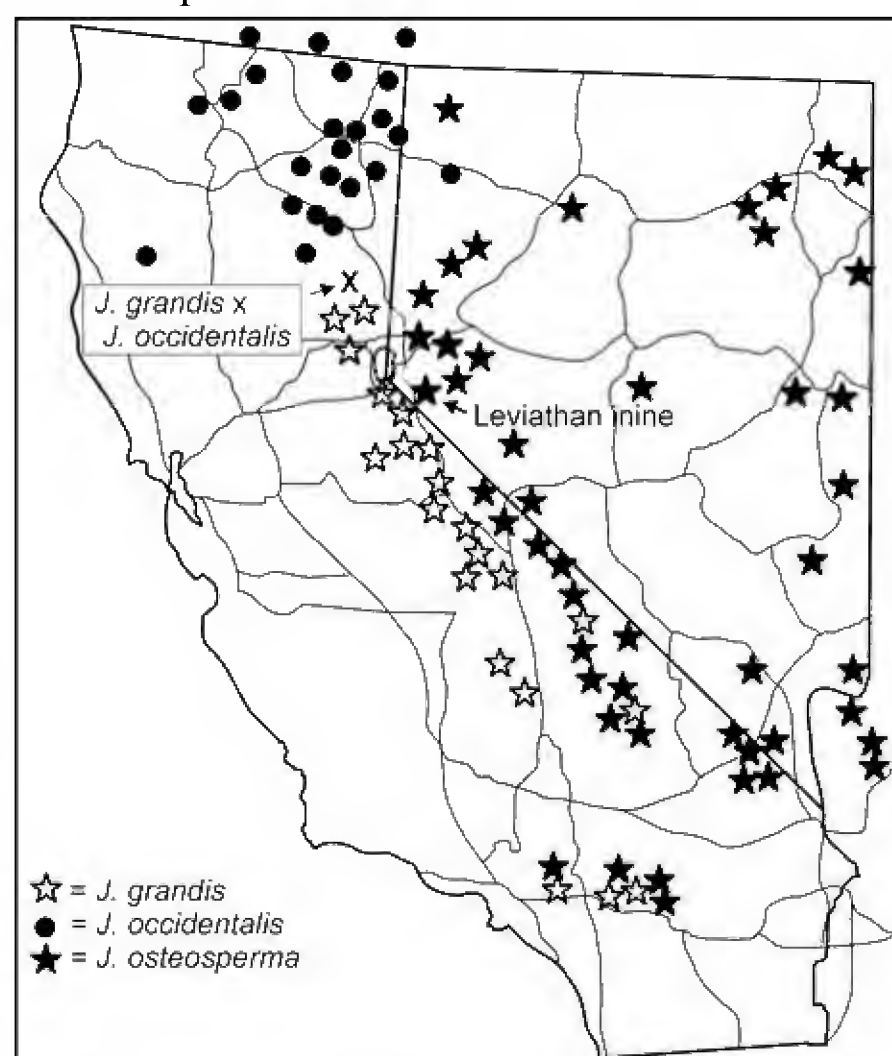


Figure 2. Distributions of *J. grandis*, *J. occidentalis* (in part) and *J. osteosperma* (in part) with Leviathan mine population noted.

B1), Yolla Bolly-Middle Eel Wilderness, 40° 06' 34" N, 122° 57' 59" W, 1815- 2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178' N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867' N, 120° 28.456' W, 1695 m, Lassen Co., CA. **Leviathan mine population:** *Adams 12368-12382*, on Leviathan Mine Rd. (= Randall Terry popn.#16), 4 mi sw of US395, 38° 46.412' N; 119° 36.268' W, 6047 ft. Voucher specimens are deposited in the herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principal components analysis (PCA) follows the formulation of Veldman (1967).

RESULTS AND DISCUSSION

Only one of the junipers in the Leviathan mine population appeared to be typical *J. grandis* (1, Table 1) and one plant appeared very similar to *J. osteosperma* (7, Table 1). The other 13 plants sampled were somewhat intermediate in morphology, but generally appeared more like *J. osteosperma*.

The oils of *J. osteosperma* are dominated by camphor (23.7%), bornyl acetate (16.6%) and sabinene (10.0%, Table 2), with moderate amounts of α -pinene, borneol and terpinen-4-ol. Whereas, typical oils of *J. grandis* and *J. occidentalis* (Table 2) have little camphor (0, 2.5%) or borneol (0, 2.2%). The oil of *J. occidentalis* has large amounts of sabinene, p-cymene, citronellol and bornyl acetate (Table 2), whereas *J. grandis* oil is dominated by δ -3-carene, α -pinene and β -phellandrene (Table 2).

The oil of tree 1, field identified as *J. grandis*, is very similar to *J. grandis* (Meyers, CA, Table 2). Hybrids 9 and 11 have some intermediated components, and generally complementary components. The oils of trees 10 and 15 are similar to *J. osteosperma*, but differ in several components. They could be backcrosses or just unusual oils of *J. osteosperma*.

As a first approximation, PCO was calculated using oils from the 15 Leviathan mine plants, *J. grandis* (10, Meyers, CA, Sonora Jct., CA), *J. occidentalis* (Mc Arthur, CA, 10) and *J. osteosperma* (8 population averages, see Materials). This PCO used character weighting (in similarities) of Fs (from ANOVA between the putative parents). The ordination clearly shows that none of the Leviathan mine plants resembles *J. occidentalis* in their terpenes (Fig. 3).

Eliminating the *J. occidentalis* plants, and running a second ANOVA between *J. grandis* and *J. osteosperma*, followed by PCO analysis, focused on differences in the oils between putative parental species, *J. grandis* and *J. osteosperma*. The first two principal components removed 62 and 6% of the

variance among samples. Ordination (Fig. 4), shows the *J. grandis* individual from Leviathan mine (1, Fig. 4) to be closely allied with typical *J. grandis*. Plants 9

Table 1. Morphological observations on plants of the Leviathan mine population.

Tree #	habit	bark color	bark exfoliation	leaf glands
1	7m tree, 3 stems, <i>J. grandis</i>	cinnamon	shaggy strips	visible, w white exudate
2	5 m shrub, 5m x 5m	gray	shaggy strips	visible, ruptured
3	7m tree, 1 stem	brown	interlaced strips	visible, few ruptured
4	6m tree, branched at 4m	gray	strips	visible, w white exudate
5	4m tree, 3 stems, twisted	gray, orange	shaggy strips	vis. only on whip lvs., few rupt.
6	6m tree, 5 stems	gray-brown	thin strips	vis., with white exudate
7	4m shrub, <i>J. osteosperma</i>	gray-brown	strips	not vis., v. few ruptured
8	3m shrub	gray	shaggy strips	vis., w white exudate
9	4m shrub-tree, 10 stems	brown	shaggy strips	vis, w white exudate
10	3m tree, 1 stem, <i>osteo</i> BC?	gray	shaggy strips	vis, very few ruptured
11	3m shrub	gray	shaggy strips	vis., w white exudate
12	3m shrub	gray-brown	shaggy strips	vis., few w clear exudate
13	3m shrub	gray	shaggy strips	vis., few w white exudate
14	5m tree, 1 stem	gray	shaggy strips	vis., not ruptured
15	1.5m shrub x 3 m, <i>osteo</i> BC?	gray	shaggy strips	few vis., v. few ruptured
<i>J. grandis</i> (typical)				
	trees, 1-3 stems	cinnamon	shaggy strips	vis., few w clear/ white exud.
<i>J. osteosperma</i> (typical)				
	shrubs, trees (1- few stems)	gray-brown	thin strips	not vis., not ruptured

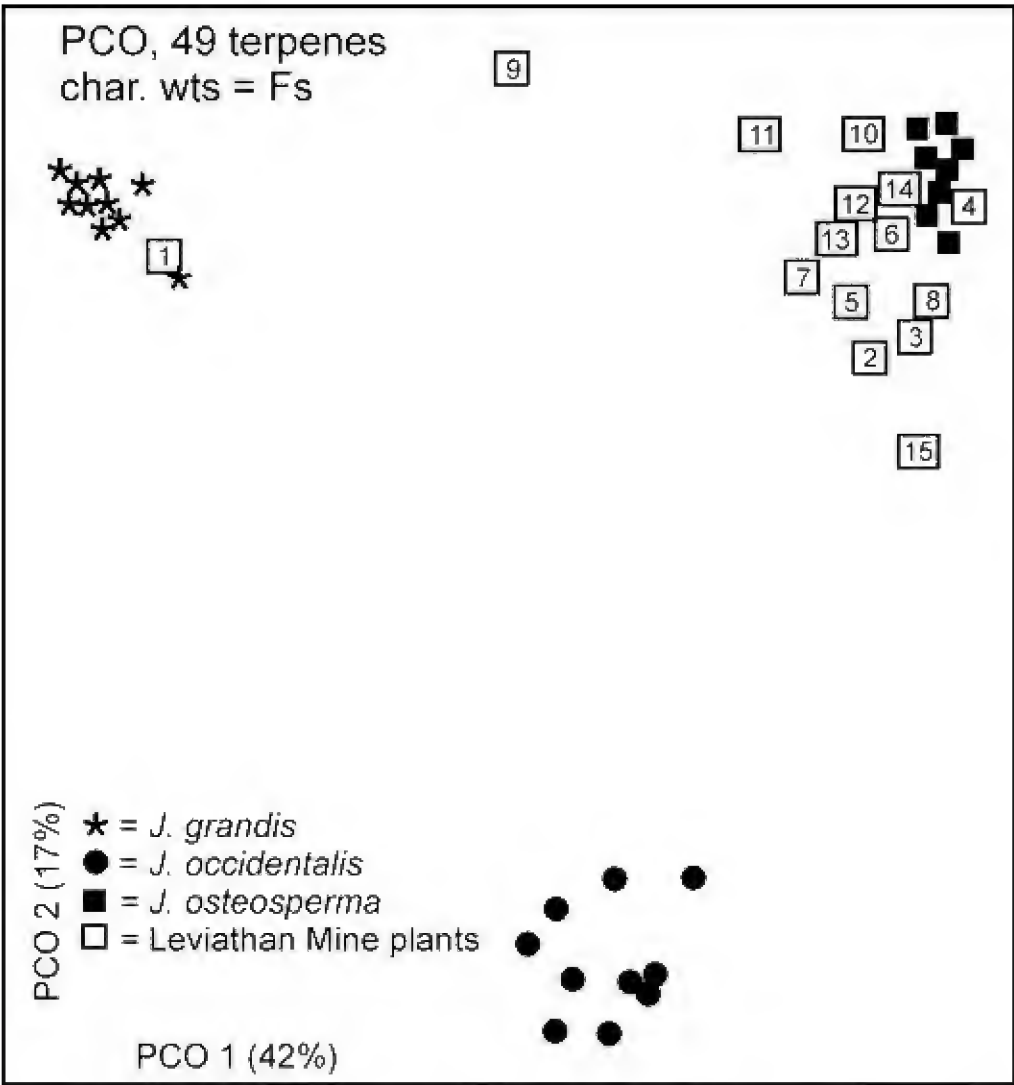


Figure 3. PCO using 49 terpenes with character weights = Fs from ANOVA between *J. grandis*, *J. occidentalis*, and *J. osteosperma*.

and 11 are intermediate and presumably hybrids. Individuals 10 and 13 are closely allied with *J. osteosperma* (Fig. 4). Nine other Leviathan mine plants are clustered between individual 11 and *J. osteosperma*.

Analysis of variation among the putative hybrids revealed that, of 32 major terpenes, 6 were intermediate (between *J. grandis* and *J. osteosperma*), 8 appeared as dominant/ recessive traits having values like one of the two species and 18 terpenes were transgressive (i.e., larger or smaller than either *J. grandis* or *J. osteosperma*). Adams and Tsumura (2012), in a study of artificial hybrids within *Cryptomeria japonica*, reported that of the 17 major terpenes, 7 were intermediate and 10 were transgressive in the F₁ hybrids. Three compounds, cedrol, widdrol and cis-thujopsene, appeared to be genetically linked and inherited as a dominant/ recessive traits with some modifying genes. This group of linked, dominant/recessive compounds interfered with the ordination of hybrids between parents, such that hybrids with large amounts of cedrol, widdrol and cis-thujopsene were very difficult to separate from the Haava parent. A second study (Adams and Stoeck, 2013) of artificial hybrids of Douglas fir (*Pseudotsuga menziesii* var. *menziesii* and var. *glauca*) found that of 19 terpenes in the F₁ hybrids, 3 were intermediate, 4 dominant/ recessive and 12 transgressive. When the 12 transgressive terpenes were truncated to values between the parents, PCO ordination was improved, with the hybrids depicted as more intermediate between the parents (Adams and Stoeck, 2013).

To investigate the effects of truncation of transgressive terpenes, the 18 transgressive terpenes were truncated to values between those of *J. grandis* and *J. osteosperma*. Extraction of eigenroots showed an increase in variance in coordinate 1 (75%) and a slight decrease in coordinate 2 (5%). Ordination shows (Fig. 5) that the overall pattern is somewhat affected. The most noticeable change is in the placement of several putative hybrids as intermediate (2, 5, 7, 12, 15, Fig. 5) and ordination of several plants towards *J. osteosperma* (3, 4, 8, 10, 13, 14, Fig. 5). The ordination in fig. 5 suggests that individuals 9

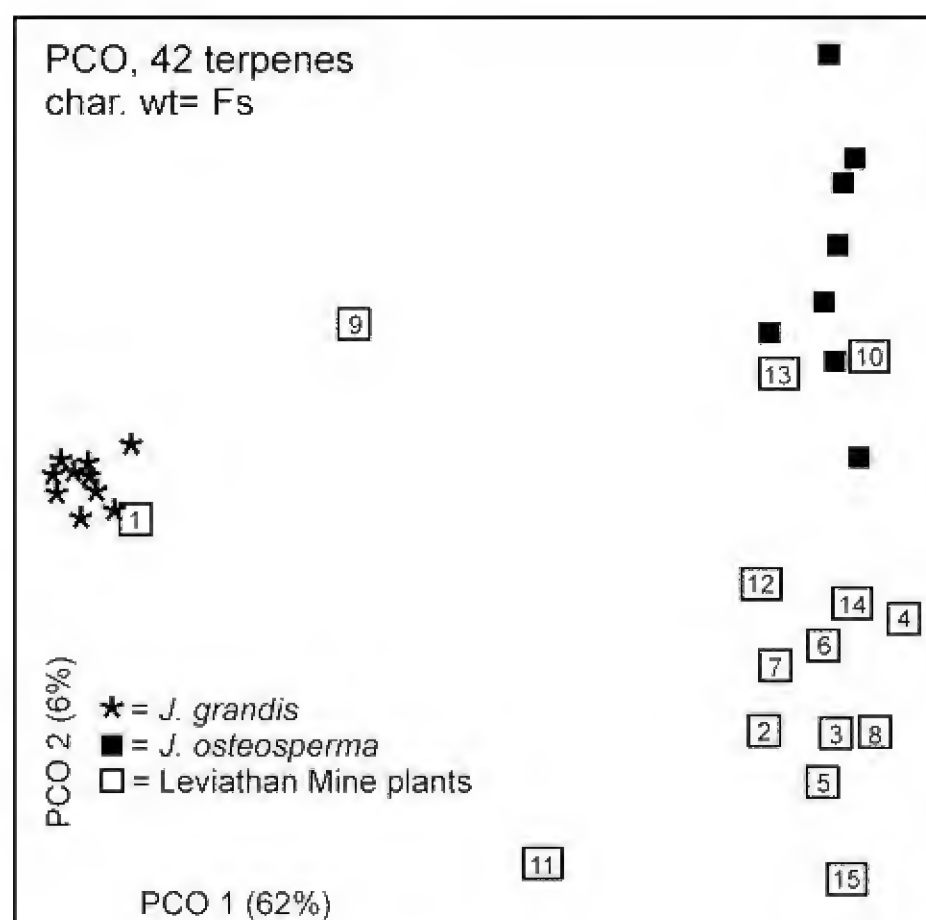


Figure 4. PCO, 42 terpenes, F weighted.

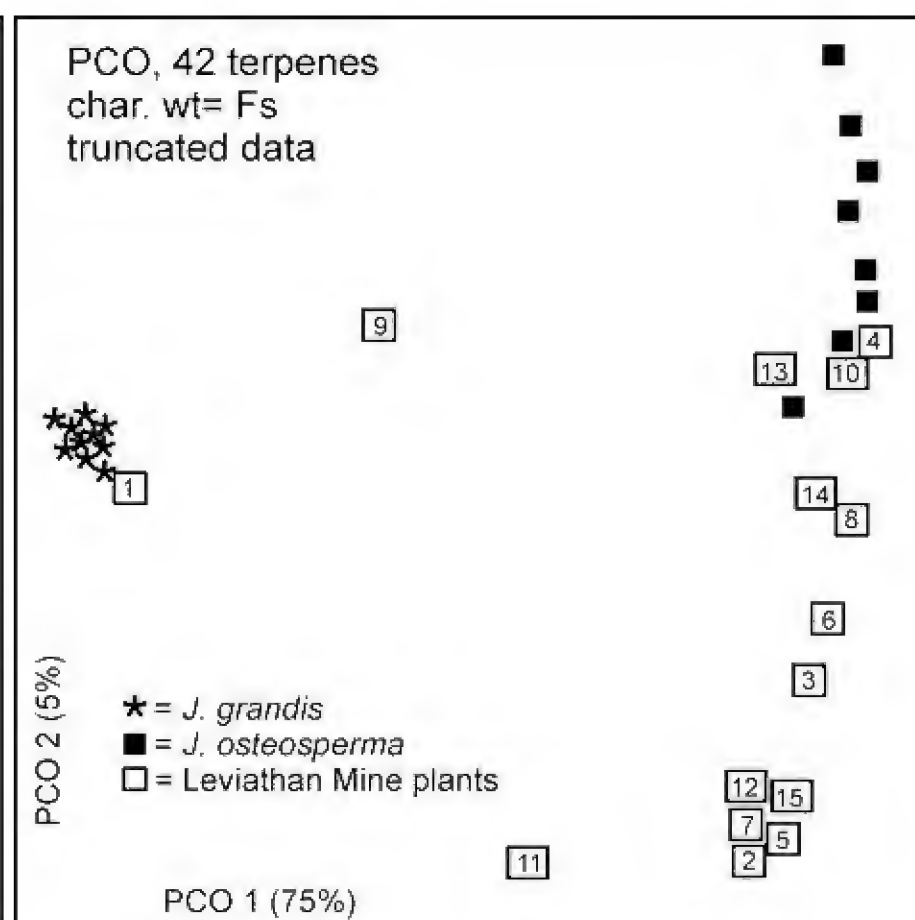


Figure 5. PCO, 42 terpenes, F weighted, data truncated between *J. grandis* and *J. osteosperma* values.

11 are hybrids with plants 2,5,7,12,15 (plus 3 and 6?) being backcrosses to *J. osteosperma*, and the remaining 5 plants are *J. osteosperma*.

Additional analyses of the variation among the Leviathan plants' terpenes was made by plotting the values along with those of *J. grandis* and *J. osteosperma*. Analyses of the 6 intermediate and 8 dominant/recessive terpenes, shows that even among those scored as intermediate (α -fenchene, verbenene, β -pinene, α -cadinol, terpinen-4-ol and borneol), many Leviathan plants had zero or trace amounts, and these low values were typical of *J. osteosperma* (Fig. 6). Only terpinen-4-ol and borneol appeared to have intermediate values (Fig. 6).

For eight dominant/ recessive compounds (3-carene, 2-carene, neo-isopulegyl acetate, KI 1092, KI 1230, trans-p-menth-2-en-1-ol, neo-isopulegol and piperitone), the Leviathan plants contained zero or trace amounts (Fig. 6). For each of the 8 compounds, the zero or trace amount is typical of *J. osteosperma*. So it is easy to see why most Leviathan plants are ordinated near *J. osteosperma* (Figs. 4, 5). Of course, it may be that most of the Leviathan plants are not hybrids, but *J. osteosperma* as suggested in Figs. 4, 5.

Analyses of the 18 transgressive terpenes found they were in 7 groups: (bornyl acetate, sabina ketone, γ -thujene, cis- and trans-sabinene hydrate, camphene hydrate), (camphor, p-mentha-1,4-dien-7-ol), (sabinene, γ -terpinene), (α -terpinene), (myrcene, KI 1154, KI 1389), (terpinolene, α -phellandrene), and (α -pinene, β -phellandrene). Several of the terpenes show extreme transgressive variation (Fig. 7, bornyl acetate, camphor, α -terpinene). Several transgressive terpenes might also be considered as dominant/ recessive traits (myrcene, terpinolene, α -pinene, Fig. 7).

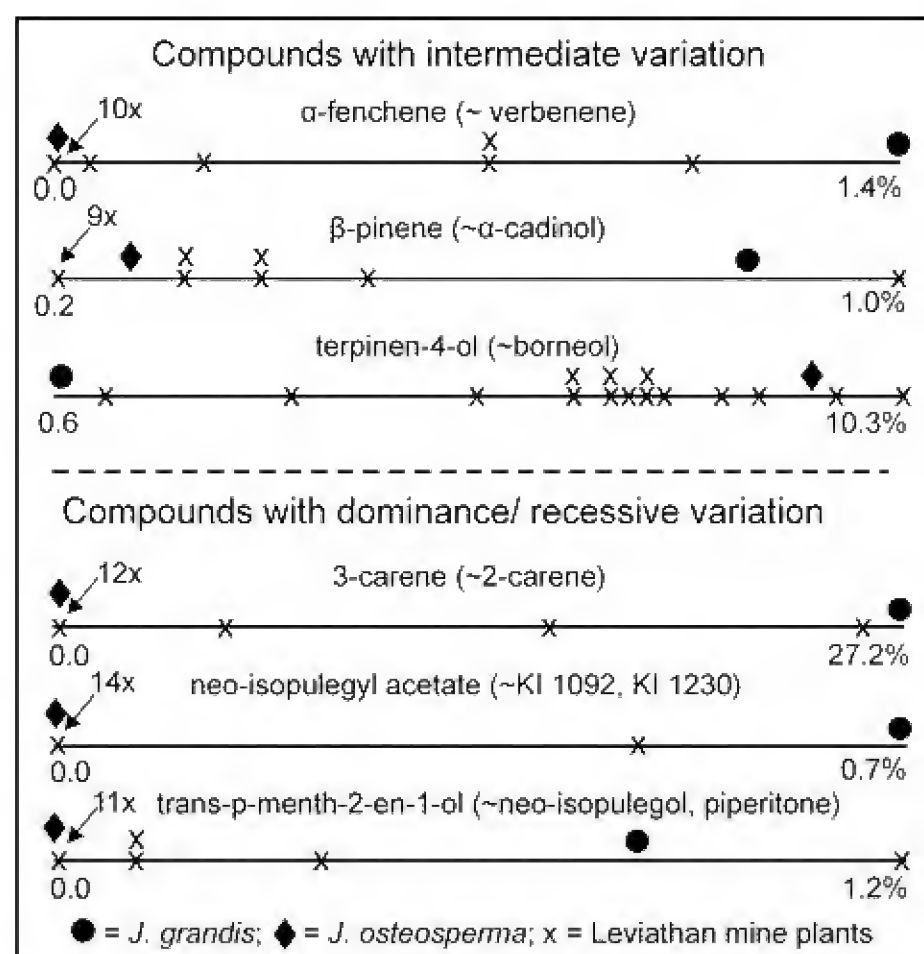


Figure 6. Terpenes with intermediate or dominant/ recessive variation.

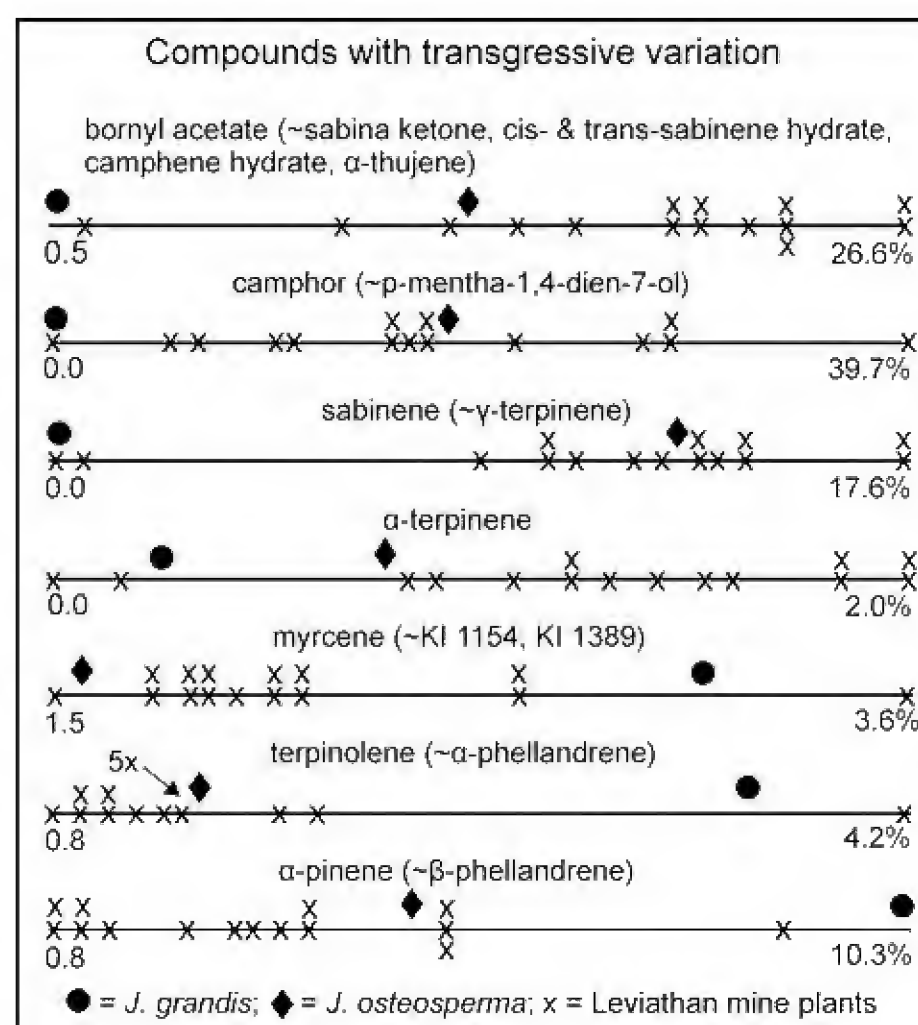


Figure 7. Terpenes with transgressive variation.

Recently, Adams and Stoehr (2013) investigated patterns of variation among Douglas fir hybrids, and reported that the parents and hybrids showed compounds that are zero or near zero in one parent were often zero in the hybrids. This pattern was unbalanced and many more terpenes had this pattern in the

inland parent than in the coastal parent. Thus, the similarities were biased towards the inland parent. Removing some of the redundant terpenes, led to a more intermediate ordination of the hybrids (Adams and Stoeck, 2013).

Analysis of the 24 terpenes with the largest Fs (from ANOVA between *J. grandis* and *J. osteosperma*) revealed that 7 are intermediate (Table 3) and their character weighting (as % total weight) ranged from 0.71% to 4.53%. Eight of the 24 appeared as dominant/ recessives with 3 compounds were more like *J. grandis* in Leviathan plants and 5 compounds were more like *J. osteosperma* in Leviathan plants (Table 3). Character weights ranged from 0.72% to 5.59%. Nine of the 24 terpenes were transgressive; 6 compounds were more like *J. osteosperma* in Leviathan plants (Table 3) and character weights ranged from 2.48% to 15.42%. To balance the number of characters that are like *J. grandis*

Table 3. Patterns of variation for the 24 terpenoids with the highest F ratios in ANOVA. Variation among *J. grandis* and *J. osteosperma* and Leviathan plants. x denotes the terpene occurrence pattern in *J. grandis*, Leviathan plants and/ or *J. osteosperma*. char wt = F, scaled as % total weight. char wt 1 is the original weighting based on 42 characters (Fs, scaled to % total), char wt 2 is the char weight based on 16 selected characters to balance modes between the parents (Fs, scaled to % total).

cpd	<i>J. grandis</i>	Leviathan	<i>J. osteosperma</i>	char wt 1	char wt 2
<u>intermediate (7)</u>					
α -terpinene	x	x	x	2.45	3.97
borneol	x	x	x	1.15	1.86
terpinen-4-ol	x	x	x	4.16	6.73
p-mentha-1,4-dien-7-ol	x	x	x	4.53	7.33
germacren-D-4-ol	x	x	x	0.71	1.14
epi- α -cadinol	x	x	x	0.77	1.25
α -cadinol	x	x	x	1.08	1.74
<u>dominant/ recessive (8), 3 cpds more like <i>J. grandis</i> in Leviathan plants, 5 cpds more like <i>J. osteosperma</i> in Leviathan plants.</u>					
α -fenchene	x	x		2.26	3.66
trans-carveol	x	x		1.44	2.34
carvone	x	x		1.97	3.19
camphene		x	x	5.59	9.05
3-carene		x	x	1.17	0
KI 1154		x	x	0.72	0
KI 1230		x	x	0.93	0
KI 1389		x	x	0.72	0
<u>transgressive (9), 6 cpds more like <i>J. osteosperma</i> in Leviathan plants.</u>					
sabinene		x	x	2.48	0
γ -terpinene		x	x	6.28	0
cis-sabinene hydrate		x	x	8.19	0
camphor		x	x	3.34	5.42
camphene hydrate		x	x	12.17	0
bornyl acetate		x	x	3.27	5.29
α -thujene	x	x	x	5.63	9.11
trans-sabinene hydrate	x	x	x	15.42	24.96
sabina ketone	x	x	x	7.98	12.92

and those like *J. osteosperma*, 4 terpenes were selected from the dominant/ recessive group and 5 were selected from the transgressive group along with the 7 intermediate terpenes, to make a set of 16 terpenes for PCO analysis. Note that char wt 2 values of zero (0) were not included in this group of 16 'selected' terpenes.

PCO based on 16 'selected' terpenes, with hybrids' values truncated and F weighted, produced an ordination (Fig. 8) that has only very small differences from PCO using 42 terpenes, truncated, and F weighted (Fig. 7). So, although this technique of balancing terpene characters between parents had a positive effect in Douglas fir (Adams and Stoeck, 2013), it does not seem to have an effect on the present data set. It might be noted that trans-sabinene hydrate has a very large percentage of the total weight (24.96%) in this analysis. The F value for trans-sabinene hydrate was changed to that of sabina ketone, so it had a much lower weight (i.e., equal to that of sabina ketone in the similarity), but only very, very minor differences were seen in the ordination.

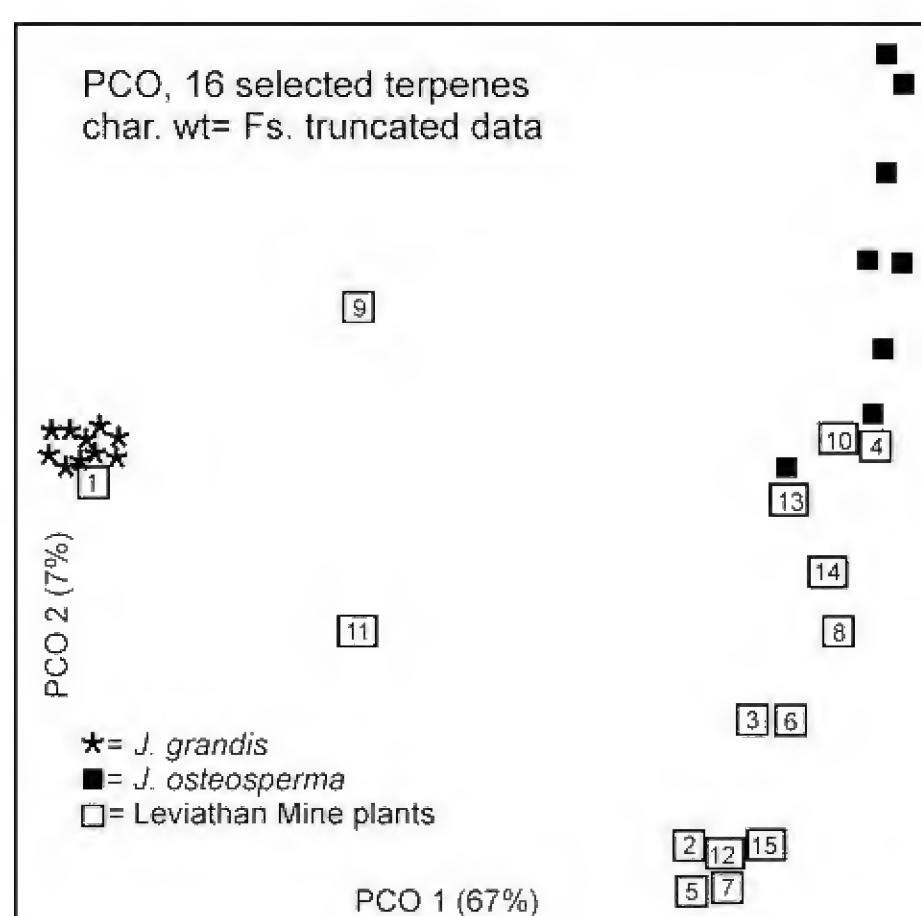


Figure 7. PCO, 16 selected terpenes, wt. = Fs, and truncated terpene values for the Leviathan plants.

giving excessive weight to some characters. The present study, using putative hybrids, mirrors the previous studies (Adams and Tsumura, 2012; Adams and Stoeck, 2013) that encountered problems with transgressive variation, linked terpenes and dominant/ recessive suites of terpenes in artificial hybrids. These problems make it difficult to accurately identify backcrossed plants.

ACKNOWLEDGEMENTS

Thanks to Billie Turner for proofing the manuscript. This research was supported with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

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CONCLUSION

It appears that the Leviathan mine population samples contain one typical *J. grandis*, 2 hybrids, 5-10 backcrossed (to *J. osteosperma*) individuals and 3 plants whose oils are fairly typical of *J. osteosperma*. The terpene data support the haplotype data of Terry (2010). It is interesting that Terry (2010) and figure 1 (above) show 5 haplotypes in the Leviathan mine population, of which only 2 of the 5 haplotypes appear in *J. osteosperma* populations (none of the 5 haplotypes appears in *J. occidentalis* populations). It seems likely that haplotypes 6, 7, and 8 are from *J. grandis* germplasm.

Finally, it should be noted that the detection of hybridization using terpenoid data and multivariate methods is subject to considerable difficulty due to the dominant/recessive and transgressive traits and genetic linkage groups

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Table 2. Leaf essential oil compositions for *J. osteosperma* (McKinney Tanks, NV) plus putative *J. osteosperma* backcrosses: #10, 15, putative hybrids, #9, 11, and putative *J. grandis*: #1, along with *J. grandis* (Meyers, CA) and *J. occidentalis* (Mc Arthur, CA). Compounds in boldface indicate hybridity.

KI	compound	osteo	#10	#15	#9	#11	#1	grand	occid
921	tricyclene	0.8	0.4	0.7	0.4	0.6	t	-	1.1
924	α -thujene	0.5	0.5	0.7	0.1	0.5	t	-	1.0
932	α -pinene	4.4	0.9	3.9	5.0	1.0	9.3	14.0	5.0
945	α-fenchene	-	-	t	0.8	-	1.1	1.5	t
946	camphene	1.1	0.6	1.1	0.7	0.8	-	-	1.0
953	thuja-2,4-diene	t	-	-	t	-	t	t	t
961	verbenene	-	-	0.2	1.3	-	0.4	2.9	-
969	sabinene	10.2	10.7	15.0	0.5	13.6	t	-	12.0
974	β -pinene	0.2	0.2	0.3	0.4	0.1	1.0	1.3	0.4
988	myrcene	1.7	1.5	2.7	2.2	1.8	3.6	3.1	1.3
1001	δ-2-carene	-	-	-	t	-	0.2	1.1	t
1002	α -phellandrene	0.3	0.3	0.2	0.4	0.1	2.4	1.6	0.8
1008	δ-3-carene	-	0.2	0.3	15.8	t	26.3	27.3	1.0
1014	α -terpinene	1.3	1.3	1.9	0.3	1.4	0.5	0.4	1.7
1020	p-cymene	2.4	1.4	0.7	3.1	0.8	1.3	1.4	10.7
1024	limonene	2.1	1.9	3.4	2.2	2.4	1.4	1.2	0.9
1025	β-phellandrene	3.2	2.9	2.2	4.4	1.5	12.4	10.6	3.5
1044	(E)- β -ocimene	t	t	0.4	0.6	0.1	t	t	0.1
1054	γ-terpinene	2.1	2.3	3.1	1.0	2.4	0.3	0.3	3.0
1065	cis-sabinene hydrate	0.8	1.3	1.4	0.1	1.6	0.3	-	0.9
1078	camphenilone	t	t	t	-	t	-	-	-
1086	terpinolene	1.4	0.9	1.4	1.9	0.9	4.2	3.7	1.3
1090	6,7-epoxymycene	0.1	-	t	-	t	-	-	-
1092	96, 109, 43, 152, C10-OH	-	-	t	0.7	-	0.3	0.9	-
1095	linalool	-	0.3	0.4	0.6	0.1	0.5	t	0.5
1098	trans-sabinene hydrate	1.0	1.4	1.5	-	1.7	-	-	-
1102	isopentyl-isovalerate	0.2	-	t	-	t	-	-	-
1112	3-me-3-buten-methyl butanoate	0.4	t	0.6	-	0.3	-	-	-
1118	cis-p-menth-2-en-1-ol	0.6	1.1	0.6	0.4	0.5	1.4	0.8	0.7
1122	α -campholenal	0.3	0.3	t	t	t	t	t	-
1136	trans-p-menth-2-en-1-ol	-	-	0.4	-	t	1.2	0.9	0.9
1141	camphor	23.7	29.5	5.5	22.9	27.8	-	-	2.5
1144	neo-isopulegol	-	-	-	-	-	0.8	0.5	-
1145	camphene hydrate	1.5	1.5	1.9	1.1	2.3	0.2	t	0.2
1154	p-menth-1,5-dien-8-ol iso.	-	-	-	-	-	1.0	0.6	-
1154	sabina ketone	0.8	1.5	0.4	1.7	0.4	-	-	0.4
1161	p-menth-1,5-dien-8-ol iso.	-	-	-	-	-	t	0.3	-
1165	borneol	6.0	1.5	2.0	1.4	0.6	-	-	2.2
1166	coahuilensol	-	-	1.6	-	-	0.4	t	0.6
1174	terpinen-4-ol	8.3	8.3	7.8	1.2	7.3	0.5	0.4	6.7
1176	m-cymen-9-ol	-	-	-	1.1	-	0.4	0.4	-
1179	p-cymen-8-ol	0.5	1.4	0.4	0.4	0.2	0.3	0.4	0.5
1186	α -terpineol	0.4	0.4	0.5	1.9	0.5	3.4	1.2	0.4
1195	myrtenol	0.2	0.2	t	t	0.3	-	-	-
1195	cis-piperitol	0.3	0.2	t	-	-	0.6	0.4	0.2
1204	verbenone	0.2	-	-	0.8	0.1	-	-	-
1207	trans-piperitol	0.3	0.3	0.3	-	0.2	1.1	0.9	0.3
1215	trans-carveol	0.6	0.4	t	-	t	-	-	-
1219	coahuilensol, me-ether	0.2	0.2	1.6	0.4	0.3	1.3	0.4	1.1
1223	citronellol	8.3	t	t	0.2	-	0.2	t	8.4
1230	trans-chrysanthenyl ac.	-	-	-	0.7	-	0.5	3.9	-

KI	compound	osteo	#10	#15	#9	#11	#1	grand	occid
1238	cumin aldehyde	0.3	0.3	0.1	-	-	-	-	0.2
1239	carvone	0.6	0.4	t	0.2	0.1	0.2	t	-
1249	piperitone	t	-	t	1.2	t	0.3	1.2	0.2
1255	4Z-decenol	-	-	-	-	-	0.6	0.4	-
1257	methyl citronellate	-	-	t	0.9	0.1	0.2	0.2	-
1274	neo-isopulegyl acetate	-	-	t	-	-	0.5	0.3	-
1283	α -terpinen-7-al	0.2	-	-	-	-	-	-	-
1284	bornyl acetate	16.6	16.8	26.3	10.0	20.3	0.9	0.4	9.5
1285	safrole	-	0.2	-	-	-	0.4	0.3	-
1298	carvacrol	t	t	0.3	0.2	0.3	t	0.2	0.4
1319	149,69,91,164, phenolic	0.4	0.6	0.7	0.5	1.9	2.5	0.4	-
1318	methyl geranate	-	-	1.1	0.5	-	0.4	0.4	1.0
1325	p-mentha-1,4-dien-7-ol	0.5	0.8	0.3	-	0.2	-	-	t
1332	cis-piperitol acetate	-	-	-	t	0.1	0.3	0.4	-
1343	trans-piperitol acetate	-	-	-	-	-	0.1	0.3	-
1387	β -bourbonene	-	-	-	-	-	t	0.5	0.2
1388	79,43,91,180	-	-	-	0.3	t	0.9	0.3	-
1389	111,81,151,182	-	-	-	1.2	0.2	3.3	1.0	-
1403	methyl eugenol	-	-	-	-	-	0.1	t	-
1429	cis-thujopsene	0.7	-	-	-	-	-	-	0.9
1448	cis-muurolo-3,5-diene	-	-	-	t	-	t	t	-
1451	trans-muurolo-3,5-diene	-	-	-	-	-	-	-	0.1
1465	cis-muurolo-4,5-diene	-	-	-	-	-	-	-	0.1
1468	pinchotene acetate	0.5	t	0.8	0.2	-	0.8	-	0.6
1471	121,105,180,208,phenol	-	-	-	-	-	-	0.3	-
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	-	-	0.3
1478	γ -muurolene	-	-	-	-	-	t	-	0.8
1484	germacrene D	-	-	-	-	-	0.2	0.2	0.3
1493	trans-murrola-4(14),5-diene	-	-	-	-	-	-	-	0.4
1493	epi-cubebol	-	-	-	-	-	t	-	0.4
1500	α -muurolene	t	-	0.2	1.0	-	0.2	0.3	1.1
1513	γ -cadinene	t	0.2	0.3	0.3	0.2	0.6	1.3	3.7
1518	epi-cubebol	-	-	-	t	t	t	0.4	0.4
1522	δ -cadinene	0.2	0.3	0.6	0.4	0.3	0.7	1.1	4.1
1537	α -cadinene	-	-	-	-	-	t	t	0.4
1544	α -calacorene	-	-	-	-	-	-	-	0.3
1548	elemol	0.9	0.6	0.1	0.5	0.7	0.2	-	-
1555	elemicin	-	-	t	-	-	0.2	1.5	-
1574	germacrene-D-4-ol	t	0.3	0.6	0.4	0.3	0.7	0.7	0.6
1582	caryophyllene oxide	t	t	t	t	-	-	t	-
1586	gleenol	-	-	-	-	-	-	-	0.3
1607	β-oplophenone	t	t	t	0.5	t	0.2	0.4	0.4
1608	humulene epoxide II	t	-	t	-	t	-	-	-
1618	1,10-di-epi-cubenol	-	-	-	-	-	t	t	0.2
1627	1-epi-cubenol	-	-	-	-	t	t	t	1.6
1630	γ -eudesmol	0.2	t	-	-	-	-	-	-
1638	epi-α-cadinol	t	0.2	0.3	0.3	0.2	0.6	0.7	1.1
1638	epi-α-muurolol	t	0.2	0.4	0.4	0.2	0.6	0.7	1.2
1644	α -muurolol	-	t	t	t	t	t	t	0.7
1649	β -eudesmol	0.2	t	-	t	0.1	-	0.4	-
1652	α -eudesmol	0.2	0.3	-	0.5	-	-	-	-
1652	α-cadinol	0.2	0.3	1.0	0.6	0.7	1.2	1.6	1.8
1688	shyobunol	-	-	0.2	-	t	0.2	0.2	-
1739	oplopanone	t	t	t	0.2	t	t	t	-
1987	manoyl oxide	-	-	t	t	-	t	t	3.2

KI	compound	osteo	#10	#15	#9	#11	#1	grand	occid
2009	epi-13-manoyl oxide	-	-	-	-	-	-	-	t
2056	manool	-	-	t	-	-	t	t	-
2055	abietatriene	-	-	t	t	-	t	t	-
2298	4-epi-abietal	-	-	t	t	-	t	t	-
2312	abieta-7,13-dien-3-one	0.1	-	-	-	t	-	-	-

KI = linear Kovats Index on DB-5 column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Additional morphological measurements of *Arceuthobium littorum* and *A. occidentale* (Viscaceae)

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ABSTRACT

Coastal dwarf mistletoe (*Arceuthobium littorum*) was separated from gray pine dwarf mistletoe (*A. occidentale*) on the basis of several morphological and physiological characteristics in 1992. However, when *A. littorum* was described, several key morphological characters had not been adequately quantified and recent molecular evidence has suggested it is closely related to several other dwarf mistletoes in California, including *A. occidentale*. Therefore, we made additional morphological measurements for *A. littorum* from eight populations along the coast of California and for *A. occidentale* from 28 populations distributed throughout its geographic range. We also made additional observations of the phenology of both taxa. Our data demonstrated that *A. littorum* was morphologically and physiologically distinct from *A. occidentale* using several characteristics of male plants: *A. littorum* had significantly larger staminate spikes, flower diameters, petal lengths and widths, and anther diameters. In contrast to *A. occidentale*, *A. littorum* produced fewer 3-merous staminate flowers, and it commonly produced 5-merous and rarely, 6-merous flowers. *Arceuthobium littorum* also consistently flowers and disperses seed approximately one month earlier than *A. occidentale*. Therefore, our data supported the classification of *A. littorum* as a distinct species from *A. occidentale*.

Published on-line: www.phytologia.org *Phytologia* 95(1):70-78 (Feb. 1, 2013).

KEY WORDS: *Arceuthobium littorum*, *Arceuthobium occidentale*, Coastal dwarf mistletoe, Gray pine dwarf mistletoe, morphology, *Pinus muricata*, *Pinus radiata*, *Pinus sabiniana*.

Arceuthobium littorum Hawksw. Wiens & Nickrent (coastal dwarf mistletoe, Viscaceae) and *A. occidentale* Engelm. (gray pine dwarf mistletoe) are parasites of pines (*Pinus* spp., Pinaceae) and are endemic to California (Hickman 1993; Hawksworth and Wiens 1996). *Arceuthobium littorum* is primarily a parasite of *Pinus muricata* D. Don (bishop pine) and *P. radiata* D. Don (Monterey pine) and is distributed along the Pacific Coast from near Fort Bragg (Mendocino County) south to Cambria (San Luis Obispo County). *Arceuthobium occidentale* is primarily a parasite of *P. sabiniana* Dougl. ex D. Don (gray pine) and is distributed in the foothills surrounding the Central Valley. These dwarf mistletoes are not sympatric, but they occur within approximately 50 km of each other in San Luis Obispo and Monterey Counties.

Prior to 1992, *A. littorum* and *A. occidentale* were considered to be conspecific (Peirce 1905, Gill 1935, Hawksworth and Wiens 1972, 1984) until Hawksworth et al. (1992) separated *A. littorum* from *A. occidentale* based on differences in plant and fruit size, host affinities, and the presence or absence of witches' brooms on infected principal hosts (see Table 3 in Hawksworth et al. 1992). Verticillate branching has also been shown to occur more commonly for *A. littorum* than *A. occidentale* (Mark and Hawksworth 1981). Using isozyme electrophoresis, Nickrent and Butler (1990) demonstrated that

populations of *A. littorum* shared a high degree of allelic identity and were genetically distinct from populations of *A. occidentale*. However, more recent molecular work using ribosomal and chloroplast DNA failed to resolve *A. littorum* and *A. occidentale* to the species level (Nickrent et al. 2004), suggesting that further study of their morphological and physiological characteristics was needed. Therefore, we initiated this study to provide more morphological and phenological data for both *A. littorum* and *A. occidentale* so that their similarities and differences could be evaluated based on additional information.

MATERIALS AND METHODS

We sampled eight populations of *A. littorum* along the Pacific Coast based on collections reported in Hawksworth and Wiens (1996) and 28 populations of *A. occidentale* throughout its reported distribution in California (Fig. 1). For each population, 10-20 male and 10-20 female infections were collected and the dominant shoot from each infection was used for morphological measurements. Measurements were made for *A. littorum* from 5 locations where it parasitized *Pinus muricata* and from 3 locations where it parasitized *P. radiata* (Fig. 1). All of the morphological measurements made for *A. occidentale* were from locations where it parasitized its principal host, *P. sabiniana*. The dwarf mistletoe plant characters measured were those used by Hawksworth and Wiens (1996) for taxonomic classification of *Arceuthobium*. The following morphological characters were measured: 1) height, basal diameter, third internode length and width, and color of male and female plants; 2) mature fruit length, width, and color; 3) seed length, width and color; 4) length and width of staminate spikes; 5) staminate flower diameter for 3- and 4-merous flowers (and 5-merous for *A. littorum*); 6) length and width of staminate flower petals; and 7) anther diameter and anther distance from the petal tip. Plants were measured within 24 hr. after collection using a digital caliper and a 7X hand lens equipped with a micrometer. Staminate spike and flower measurements were made during the peak of anthesis, and fruit and seed measurements were made during the peak of seed dispersal. Voucher specimens representing all of the populations illustrated in Fig. 1 have been deposited at the University of Arizona Herbarium (UA).

Because the times of flowering and seed dispersal for *A. littorum* were poorly known (Hawksworth and Wiens 1996), additional observations of its phenology were made during the summer and fall of 2010-2012. Additional observations of the phenology of *A. occidentale* were made during the summer and fall of 2007-2012.

RESULTS AND DISCUSSION

Although Hawksworth et al. (1992) reported that the mean plant heights for *Arceuthobium littorum* and *A. occidentale* were distinctly different (12 and 8 cm, respectively), we found the mean heights of the plants were nearly identical when comparing male and female plants together (10.4 cm). Mean plant heights also varied between the male and female plants, but the differences were not statistically significant (Table 1). The most conspicuous difference between the plants of *A. littorum* and *A. occidentale* was their shoot color. While color is often an unreliable and/or less informative character for distinguishing dwarf mistletoes, the shoot color of these species was markedly different. Plants of *A. littorum* were consistently dark green, brown-green, or sometimes yellow-brown, while those of *A. occidentale* were glaucous and yellow, yellow-green, or straw. Plants of *A. littorum* were rarely glaucous and when it was observed it was a very thin waxy coating and present only near the base of plants. In contrast, plants of *A. occidentale* were frequently glaucous over their entire length, as reported by Hawksworth and Wiens (1996).

The mean basal diameter of plants was larger for *A. littorum* (3.5 mm) than for *A. occidentale* (3.1 mm), but not as great as the difference for this character reported by Hawksworth et al. (1992) (3.4 versus 2.3 mm). While Hawksworth et al. (1992) only reported a maximum basal diameter of 4 mm for

A. occidentale, we measured plants of both sexes that had basal diameters ≥ 6 mm which resulted in a larger mean basal diameter for the populations we sampled. Nevertheless, the mean basal diameter of the plants we measured for *A. littorum* (both sexes combined) was significantly different from the mean basal diameter for *A. occidentale*. In Table 1, we present the mean basal diameter of plants by sex and these values were also significantly different.

Because Hawksworth et al. (1992) reported the length and width of the third internode for the plants they measured, we also measured these dimensions. Our measurements indicated that the mean length and width of the third internode of *A. littorum* were significantly larger than those of *A. occidentale* for plants of both sexes (Table 1). The use of third internode dimensions as a taxonomic character, however, is not accepted by all investigators who have studied dwarf mistletoes. Kuijt (1970) demonstrated that dwarf mistletoes have basal meristems, and therefore, internodes may elongate for many years. This led Kuijt (1970) to discount the use of third internode dimensions, and we agree that the length of third internodes should not be used as a key character to separate taxa of *Arceuthobium*. However, we have continued to use the dimensions of the third internode for comparing dwarf mistletoes because the width of the third internode provides a relative measure of the robustness of plants beyond that of only using their basal diameter. Combining measurements of basal diameter and the width of the third internode provides a quantitative method of comparing the overall shoot thickness of the plants between species. Because Hawksworth and Wiens (1972, 1996) have long used the third internode for their studies of dwarf mistletoe morphology, we have followed this convention.

Although the length and width of staminate spikes is extremely variable for most species of dwarf mistletoes, staminate spike morphology can be taxonomically informative when it is quite distinct from other species. For example, staminate spikes of *Arceuthobium strictum* Hawksw. & Wiens are distinctive in that they often don't branch and may be as long as 6-13 cm (Hawksworth and Wiens 1996). We noted a similar characteristic for *A. littorum*, in that staminate spikes were often un-branched and were sometimes nearly 6 cm long. Moreover, some male plants emerging from an infected branch did not branch and were almost 5 cm in height. We did not observe this pattern of staminate spike production for *A. occidentale*, but some staminate spikes of this species that diverged off of the main shoot were unbranched and reached lengths of over 3 cm. However, this growth pattern for staminate spikes of *A. occidentale* was rare and most of its staminate spikes were about 1-2 cm long. The mean length of staminate spikes was significantly larger for *A. littorum* than *A. occidentale* (20.6 versus 13.9 mm) (Table 1) and staminate spikes > 3 cm in length were commonly formed on male plants of *A. littorum*. In addition, the mean width of staminate spikes of *A. littorum* (3.4 mm) was significantly greater than the mean width of staminate spikes of *A. occidentale* (2.9 mm) (Table 1).

A major difference found between *A. littorum* and *A. occidentale* that has previously been unreported, was that *A. littorum* frequently develops staminate flowers with 5 petals, and rarely, flowers with 6 petals. Although *A. littorum* predominantly produced 4-merous staminate flowers, as reported by Hawksworth et al. (1992), it also produced 3-merous flowers, but less often than 5-merous flowers. A survey of 100 flowers in separate populations of *A. littorum* indicated that it produced about 15% 3-merous, 60% 4-merous, and 25% 5-merous flowers. Only one 6-merous flower was found during our survey of petal numbers and this configuration was only rarely observed while we were measuring morphological characters for staminate flowers. Staminate flowers of *A. occidentale* were predominantly 3- or 4-merous, but we did observe one 5-merous flower while measuring staminate flower dimensions; no 6-merous flowers were observed on staminate plants of *A. occidentale*. The relatively low frequency of 3-merous flowers and frequent formation of 5-merous flowers by *A. littorum* clearly distinguished it from *A. occidentale*. Furthermore, *A. littorum* consistently formed larger 3- and 4-merous flowers, with significantly longer and wider petals than *A. occidentale* (Table 1). *Arceuthobium littorum* also produced significantly larger anthers, some which were 1.5 mm in diameter (Table 1). The largest anther diameter measured for *A. occidentale* was 1 mm.

Although Hawksworth et al. (1992) reported that the fruits of *A. littorum* were slightly longer than those of *A. occidentale*, our measurements of mature fruits indicated they were approximately the same length (means and ranges), but that the average width of fruits of *A. littorum* was greater than for *A. occidentale* (Table 1). However, the largest fruits of *A. occidentale* measured were over 4.5 mm in width, while those of *A. littorum* did not exceed 4.4 mm. Therefore, the dimensions of mature fruits cannot be used to distinguish these dwarf mistletoes from each other, nor can the dimensions of mature seeds (Table 1). A clear distinction between the fruits of these two taxa was their color and whether they were glaucous or non-glaucous. Fruits of *A. littorum* were consistently dark green and sometimes red, but only lightly glaucous, if at all. In contrast, fruits of *A. occidentale* were light green and remarkably glaucous to the point of appearing blue. Removal of the waxy coating on the fruits of *A. occidentale* revealed their light green color, but the fruits of *A. littorum* were distinctly dark green and remained so when any wax on the fruit surface was removed.

Flowering of *A. littorum* started in late August, peaked in mid to late September, and ended by mid October. In contrast, *A. occidentale* did not start flowering until early October, peaked in late October or early November, and did not complete flowering until mid December. Fruit maturation of *A. littorum* also occurred earlier than for *A. occidentale*. Fruits of *A. littorum* started seed dispersal in early September, peaked in late September and early October, and completed this process by late October during 2010. However, we observed some seed dispersal in early November 2011 and Peirce (1905) reported that seeds of *A. littorum* (classified then as *A. occidentale*) were still dispersing around Christmas in 1903. Therefore, additional observations of seed dispersal of *A. littorum* are warranted to better define when it is completed. Seed dispersal of *A. occidentale* didn't start until early October, peaked in late October to early November, and consistently continued into December. Therefore, flowering and seed dispersal of *A. littorum* usually started approximately one month before *A. occidentale* and was completed about one month earlier, but as mentioned above, additional observations of seed dispersal are needed for *A. littorum* because of the report by Peirce (1905).

Another distinction between *A. littorum* and *A. occidentale* reported by Hawksworth et al. (1992) is that the former species consistently induces large, non-systemic witches' brooms on its pine hosts, while the latter species does not. We also observed this characteristic of broom formation on *Pinus muricata* and *P. radiata* by *A. littorum* and only rarely observed broom formation on *P. sabiniana* infected by *A. occidentale*. The reasons for the rare formation of witches' brooms on *P. sabiniana* remain unclear, but it is evidently related to a specific physiological interaction between *A. occidentale* and *P. sabiniana* because we observed that *A. occidentale* did induce the formation of brooms on its less frequently infected pine hosts such as *P. ponderosa* Dougl. ex Lawson & C. Lawson and *P. coulteri* D. Don.

Although the pine hosts of these dwarf mistletoes differ, little is known about host susceptibility because the distribution of *A. littorum* does not overlap with the distribution of the pines' hosts of *A. occidentale* and vice versa. Scharpf (1969) successfully inoculated *Pinus radiata* with *A. occidentale*, and this host-mistletoe combination has been reported from near Mount Hamilton, CA where ornamental *P. radiata* was planted near *P. sabiniana* infected with *A. occidentale* (Hawksworth and Wiens 1996). But the susceptibility of the pine hosts of *A. occidentale* to *A. littorum* remains unknown, as does the susceptibility of *P. muricata* to *A. occidentale*. Therefore, an artificial cross inoculation study using seeds of *A. littorum* on the hosts of *A. occidentale*, and vice versa, should provide useful information on the host specificity of these dwarf mistletoes.

CONCLUSIONS

Although these dwarf mistletoes were considered to be conspecific until 1992, there are several morphological and physiological characters that support their classification at the specific level (Table 2). While color is not generally considered a reliable character to use for distinguishing dwarf mistletoes, particularly in the United States (Hawksworth and Wiens 1996), these two taxa can easily be distinguished by plant color, even if the plants are distinctly glaucous or not (Table 2). Because the dimensions of female plants, mature fruits, and seeds were similar for these species, the characters of female plants are not useful for distinguishing between *A. littorum* and *A. occidentale*. The most reliable characters to use for distinguishing these species are associated with male plants. The staminate spikes of *A. littorum* are frequently unbranched and are often much longer (> 3 cm) and thicker than those of *A. occidentale* (Table 2). Furthermore, while both species produce 4-merous staminate flowers, *A. littorum* frequently produces 5-merous flowers (ca. 25%), 3-merous flowers occasionally (ca. 15%), and rarely forms 6-merous flowers ($< 1\%$); whereas *A. occidentale* produces 3- and 4-merous flowers in approximately equal proportions, and very rarely forms 5-merous flowers. The frequent formation of 5-merous flowers by *A. littorum* clearly delimits it from *A. occidentale* and other dwarf mistletoes in California. The formation of 5-merous flowers has only been reported for *A. blumeri* A. Nelson from southern Arizona and northern Mexico and *A. strictum* from northern Mexico (Hawksworth and Wiens 1996). Another important difference between *A. littorum* and *A. occidentale* is that the flowers of *A. littorum* are significantly larger in diameter, have larger petals and larger anthers. The phenology of the two species also separates them because *A. littorum* flowers and disperses seed about one month earlier than *A. occidentale*. Therefore, we agree with Nickrent and Butler (1990), Hawksworth et al. (1992), and Hawksworth and Wiens (1996) that *A. littorum* and *A. occidentale* are distinct species and should continue to be recognized as such. These mistletoes are clearly defined by both morphological and physiological differences that support their classification at the specific rank.

ACKNOWLEDGEMENTS

Reviews by Drs. Greg Filip, USDA Forest Service, Portland, OR and Shawn Kenaley, Cornell University, Ithaca, NY are sincerely appreciated.

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Table 1. Morphological measurements for *Arceuthobium littorum* and *A. occidentale*. Data are listed as **mean** (range) [n]. Means followed by different capital letters in the same row were significantly different (ANOVA followed by a Tukey's Honestly Significant Differences post hoc test, $\alpha \leq 0.05$). Lower case letters in brackets designate sample sizes already listed in the same column. Plant heights are in cm and all other measurements are in mm.

Character	<i>Arceuthobium littorum</i>	<i>Arceuthobium occidentale</i>
Plant Height		
Female	10.3 A (5.1-18.7) [100a]	10.6 A (4.9-23.2) [280a]
Male	10.5 A (5.4-22.8) [a]	10.1 A (4.7-24.1) [a]
Basal Diameter		
Female	3.4 A (2.4-6.9) [a]	3.2 B (1.7-6.0) [a]
Male	3.5 A (2.5-5.8) [a]	3.0 B (1.8-6.4) [a]
Length of Third Internode		
Female	13.7 A (7.1-23.6) [a]	12.0 B (4.6-20.6) [a]
Male	13.5 A (6.9-19.6) [a]	11.7 B (4.8-21.3) [a]
Width of Third Internode		
Female	2.6 A (1.9-3.7) [a]	2.2 B (1.3-3.5) [a]
Male	2.7 A (1.8-3.6) [a]	2.2 B (1.3-3.8) [a]
Staminate Spike Length	20.6 A (6.1-55.9)[a]	13.9 B (6.2-33.9) [200b]
Staminate Spike Width	3.4 A (2.1-4.2) [a]	2.9 B (2.2-4.1) [b]
Mean Flower Diameter		
3-merous	3.5 A (2.2-4.8) [50]	3.0 B (2.2-4.1) [185c]
4-merous	5.2 A (3.4-6.9) [135]	4.1 B (3.0-6.2) [c]
5-merous	5.7 (4.6-7.0) [20]	Only one 5-merous flower observed
Perianth Length	1.9 A (1.0-2.8) [205b]	1.5 B (1.1-2.5) [370d]
Perianth Width	1.6 A (0.8-2.5) [b]	1.3 B (0.7-2.2) [d]
Anther Diameter	0.9 A (0.4-1.5) [b]	0.6 B (0.4-1.0) [d]
Anther Distance from Tip	0.9 A (0.4-1.7) [b]	0.6 B (0.2-1.2) [d]
Mean Fruit Length	5.3 A (4.1-6.4) [a]	5.2 A (3.9-6.8) [220e]
Mean Fruit Width	3.6 A (2.9-4.4) [a]	3.3 B (2.4-4.7) [e]
Seed Length	3.4 A (2.5-4.2) [a]	3.4 A (2.5-4.3) [e]
Seed Width	1.3 A (1.0-1.6) [a]	1.3 A (1.0-1.7) [e]

Table 2. Principal morphological and physiological characteristics distinguishing *Arceuthobium littorum* from *Arceuthobium occidentale*. Numerical values are means, except staminate spike length.

Character	<i>Arceuthobium littorum</i>	<i>Arceuthobium occidentale</i>
Basal Diameter ^a (mm)	3.5	3.1
Plant Color	Brown-green, dark green, yellow-brown	Yellow, yellow-green, straw
Plants Glaucous	Rarely	Usually
Staminate Spike Length	Frequently > 3 cm	Usually < 3 cm
Staminate Spike Width (mm)	3.5	2.9
Flower Diameter (mm)		
3-merous	3.5	3.0
4-merous	5.2	4.1
5-merous	5.7	- ^b
Flowers with 3 petals	Occasional	Common
Flowers with 5 petals	Common	Rare
Flowers with 6 petals	Rare	None observed
Perianth Length (mm)	2.0	1.5
Perianth Width (mm)	1.6	1.3
Anther Diameter (mm)	0.9	0.6
Anther Distance from Tip (mm)	0.9	0.6
Fruit Color	Dark green to red	Light green
Fruits Glaucous	Lightly glaucous	Highly glaucous

^a – Male and female plants combined.

^b – Only one 5-merous flower observed.

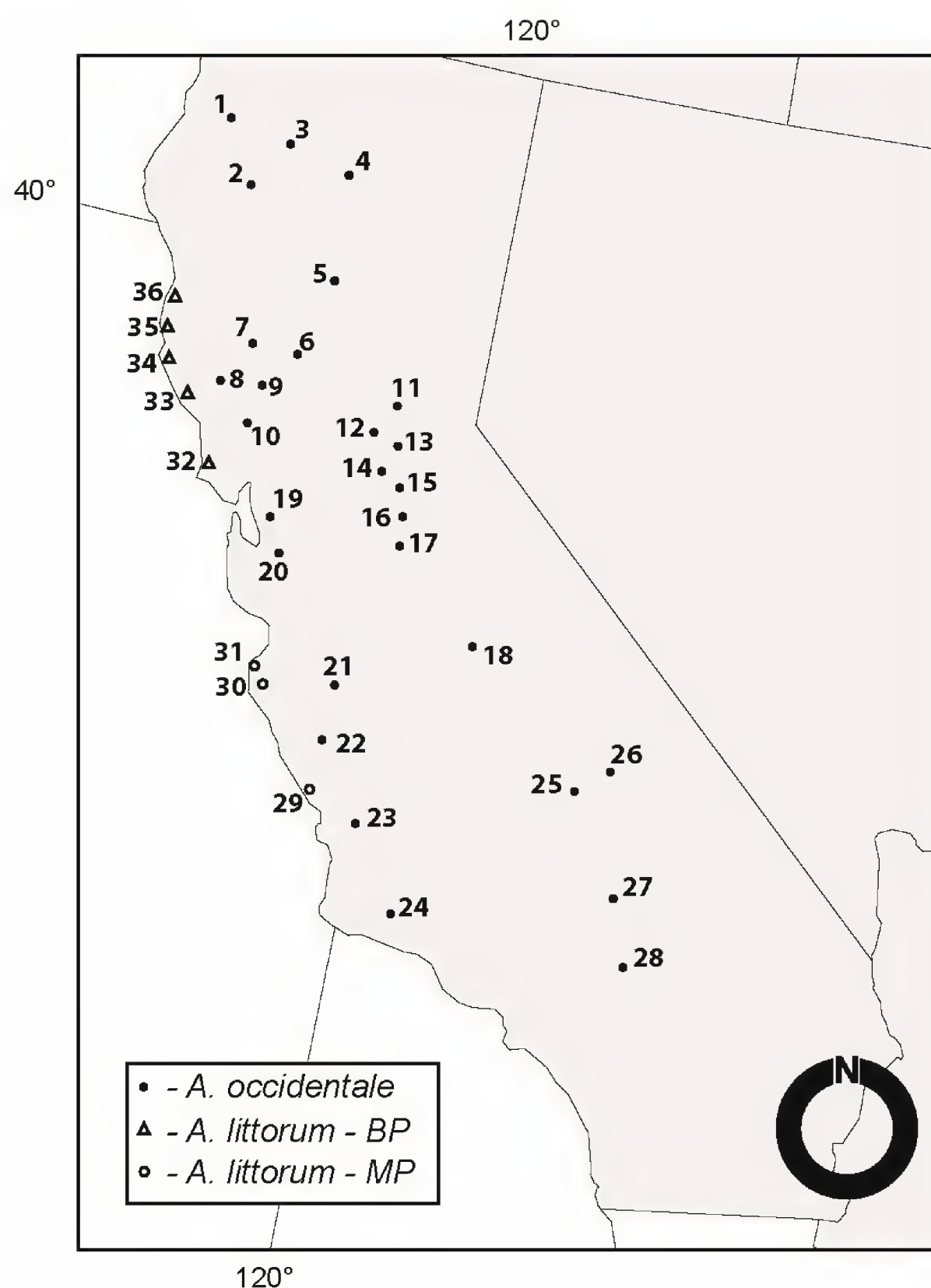


Figure 1. Approximate location of collection sites in California. Dark circles represent locations where *A. occidentale* was collected and measured on *Pinus sabiniana*. Open circles represent locations where *A. littorum* was collected and measured on *Pinus radiata* (MP). Open triangles represent locations where *A. littorum* was collected and measured on *Pinus muricata* (BP). Numbers correspond to the following locations: 1 – 2 km S of St. Rte. 299 on Burnt Ranch School rd.; 2 – Beegum Creek on St. Rte. 36; 3 – 1 km S of St. Rte. 299 on Carr Powerhouse rd.; 4 – 29 km E. of Redding on St. Rte. 44 at Black Butte rd.; 5 – 14 km NE of St. Rte. 99 on St. Rte. 70; 6 – 0.5 km E of Colusa County line on St. Rte. 20; 7 – 3 km from St. Rte. 20 on County Rd. M-12; 8 – 3 km S of Covelo on St. Rte. 162; 9 – Entrance to Langtry Winery; 10 – Butts Canyon; 11 – 1 km S of Auburn on St. Rte. 49; 12 – Beales Pt. Campground on Folsom Lake; 13 – 4 km N of Placerville on St. Rte. 49; 14 – 6 km S of St. Rte. 16 on St. Rte. 124; 15 – Columbia Airport rd.; 16 – 11 km S of

Angels Camp on St. Rte. 4; 17 – N side of Roberts Memorial Bridge on St. Rte. 120; 18 – 0.2 km SW of Prather on Auberry Rd.; 19 – 3 km N of entrance gate to Mount Diablo State Park; 20 – 19 km E of San Jose on Mount Hamilton rd.; 21 – 5 km W of visitors center in Pinnacles Nat. Mon.; 22 – 10 km E of Jolon on Nacimiento-Ferguson rd.; 23 – 5 km SE of St. Rte. 58 on Pozo rd.; 24 – 13 km E of Los Olivos on Figueroa Mt. rd.; 25 – 13 km E of Glenville on St. Rte. 155; 26 – 3 km S of Kernville; 27 – 1 km SW of St. Rte. 58 on Hart Flat rd.; 28 – 1 km S of Lake Hughes on Lake Hughes rd; 29 – 1 km N of Cambria on Santa Rosa Cemetery rd.; 30 – 0.5 km E of St. Rte. 1 on Fern Canyon rd.; 31 – Pacific Grove, 0.5 km E of Stevenson Dr. on Forest Lake rd.; 32 – 3 km NW of Inverness, Pt. Reyes Nat. Seashore; 33 – 0.5 km E of St. Rte. 1 on Kruse-Rhododendron rd.; 34 – 7 km E of Pt. Arena on Eureka Hill rd.; 35 – 4 km E of Albion River on Little River rd.; 36 – 4 km E of Fort Bragg on St. Rte 20.

Haploesthes hintoniana* (Asteraceae: Tageteae), A new gypsophilic species from Coahuila, Mexico*Billie L. Turner**

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ABSTRACT

A novel gypsophilic taxon, ***Haploesthes hintoniana*** B.L. Turner, **sp. nov.**, is described from southwestern Coahuila, Mexico. It is seemingly most closely related to the allopatric gypsophile, *H. greggii*, a commonly encountered, widespread, species of north-central Mexico and adjacent U.S.A. A photograph of the Type is provided, along with a map showing distributions of the taxa concerned. Published on-line: www.phytologia.org *Phytologia* 95(1): 79-82(Feb. 1, 2013).

KEY WORDS: Asteraceae, Tageteae, *Haploesthes hintoniana*, Mexico.

Preoccupation with the identification of Mexican Asteraceae has occasioned the present paper. I follow Panero (2007) in positioning *Haploesthes* in the tribe Tageteae, this based upon DNA data.

HAPLOESTHES HINTONIANA B.L. Turner, **sp. nov.** **Fig. 1**

Suffruticose, glabrous, succulent, herbs or shrublets, 30-70 cm high. **Leaves** opposite, linear, glabrous, connate at very base, 4-8 cm long, 1-2 mm wide. **Capitulescence** a terminal array of 5-10, fasciculate heads, the ultimate peduncles 1-2 bracteate, 6-10 mm long. **Heads** campanulate, 5-6 mm high, 3-4 mm wide; involucral bracts (outer) 5, 5-6 mm long, 1.5-2.0 mm wide, glabrous, broadly ovate, their apices obtuse to rounded, somewhat keeled at base. **Receptacle**, convex, 2-3 mm across, epaleate, glabrous. **Ray florets**, 5, pistillate, fertile; ligules, 3-5 mm long, bright yellow. **Disc florets** ca 20 per head; corollas ca 5 mm long, 5-lobed, yellow, glabrous. **Achenes**, ca 1.5 mm long, hispid throughout; pappus of ca 40 white, delicate bristles, 3-4 mm long.

TYPE: MEXICO. COAHUILA: Mpio, Francisco 1 Madero, "West side of Valle de Buenavista Francisco 1. Madero," 1835 m, Gypsum hillside, 20 Sep 2012, 26 34 18 N, 103 02 17.6 W, *Hinton et al.* 29349 (Holotype: MEXU; isotypes: GBH, TEX).

According to its collector, *H. hintoniana* was the dominant plant at the gyp site concerned (Figs. 2, 3); the novelty is named for the Hinton family.

ADDITIONAL COLLECTIONS EXAMINED: MEXICO. COAHUILA: essentially same locality as Type, 5831 ft, 26 36 43.1 N, 103 02 23.9 W, 22 Aug 2012, *Moore et al.* 2001 (LL-TEX); ca 1 km E of hwy from Finisterre to Quimicas del Rey, 3768 ft, 26 39 14.4 N, 103 08 38.4 W, 25 Aug 2012, *Moore et al.* 2070 (LL-TEX).

In my seminal treatment of *Haploesthes* (Turner 1975), this novelty will key to *H. greggii* var. *texana* (Coulter) I.M. Johnst., so far as known a taxon restricted to Texas; it differs from the latter in having more nearly ovate (vs oval), mostly longer involucral bracts (5-6 mm long vs 3-4 mm), fewer disc florets (ca 20 per head vs 60-100), and markedly pubescent achenes with spreading hairs (vs sparsely pubescent with appressed hairs).

The geographical relationship of *H. hintoniana* to yet other taxa of *Haploesthes* is shown in Map 1.

ACKNOWLEDGEMENTS

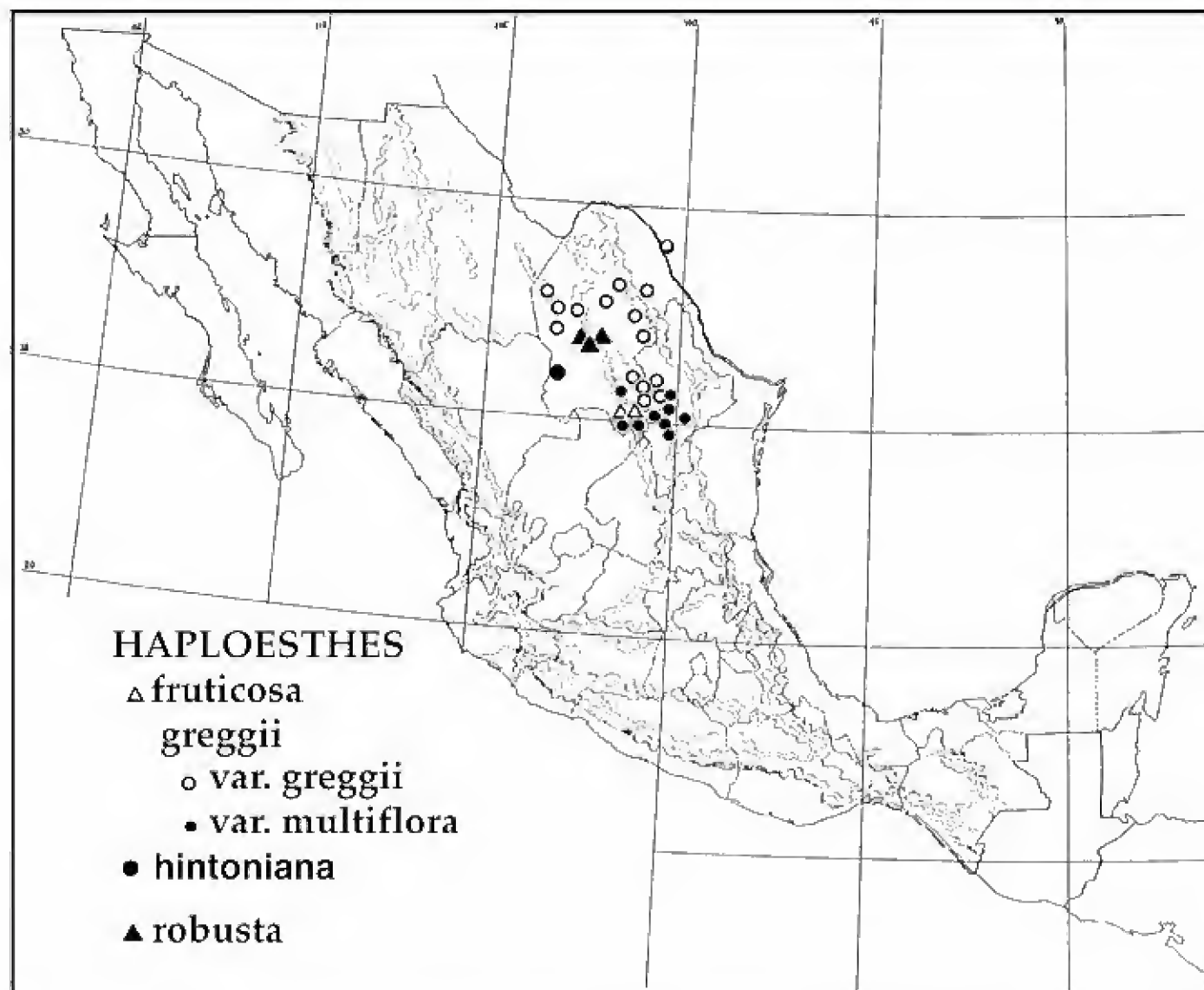
I am grateful to George S. Hinton and Prof. Mike Moore for calling my attention to the novelty, both providing photographs of the plant as it grows in the field, and to Jana Kos for providing editorial assistance. Figures 3 and 4, courtesy of Mike Moore.

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Figure 1. *Haploesthes hintoniana* (Holotype: MEXU).



Map 1. Distribution of *Haploesthes* spp. in Mexico.



Figure 2. *Haploesthes hintoniana*, in the field at the type locality (Hinton photograph).



Figure 3. *Haploesthes hintoniana*, on gypsum in field (Moore photograph).



Figure 4. *Haploesthes hintoniana*, close up of heads (Moore photograph).

Two new species of *Bartlettina* (Asteraceae: Eupatorieae) from Oaxaca, Mexico

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ABSTRACT

Two new taxa of *Bartlettina* from Oaxaca are described: ***Bartlettina solavegana* B.L. Turner, sp. nov.** and ***Bartlettina textitlana* B.L. Turner, sp. nov.** Photographs of their types are provided, along with a map showing their distribution. Since the treatment of *Bartlettina* for Mexico (Turner (1997)), three additional taxa have been added to the assemblage (Turner 2010), and the two proposed herein, all from Oaxaca; the later state is now known to have at least 14 species.

Published on-line: www.phytologia.org *Phytologia* 95(1): 83-86 (Feb. 1, 2013).

KEY WORDS: Asteraceae, Eupatorieae, *Bartlettina*, Mexico, Oaxaca

Routine identification of Mexican comps, especially those garnered in the state of Oaxaca by SERBO, has occasioned the present paper.

BARTLETTINA SOLAVEGANA B.L. Turner, sp. nov. Fig. 1.

Shrubs to 2 m high. **Stems** (upper) densely puberulent with softly-matted, slender, hairs, the vestiture ca 0.5 mm high. **Leaves** opposite throughout, 20-25 cm long, 8-9 cm wide; petioles 3-5 cm long; blades pinninervate, widest near the middle, the upper and lower surfaces glabrous or nearly so, the margins irregularly serrate. **Capitulescence** a terminal, cymose panicle ca 15 cm high and as wide, composed of numerous heads, the ultimate peduncles 3-10 mm long, puberulent like the stems. **Heads** 7-8 mm high, 8-9 mm wide; involucre bracts imbricate, 3-4 seriate, the inner series lanceolate, ca 7 mm long, 1 mm wide. **Receptacle**, 2-3 mm across, plane, glabrous. **Florets** ca 80 to a head; corollas pink, ca 5 mm long, glabrous; lobes 5, ca 1 mm long. **Achenes** ca 2 mm long, glabrous; pappus of ca 50 stiffly white bristles ca 5 mm long.

TYPE: MEXICO. OAXACANA: Distrito Sola de Vega, Mpio. Santiago Textitlan, “A u lado de la Hierba Santa.” ca 1708 m, 16 39 53.6 N, 97 16 10.2 W, 19 Feb 2007, *Maria Ester Jacob Salinas* 1647 (Holotype MEXU; isotype LL-TEX).

In my treatment of the *Bartlettina* complex of Mexico (Turner 2010), this novelty will key to *B. serboana* B.L. Turner which it resembles in habit and leaf morphology, but having much smaller heads (involucre ca 4 mm high, vs 7 mm) and fewer florets to a head (ca 15, vs ca 80), and glabrous corollas (vs pubescent).

Name derived, in part, from the Distrito Sola de Vega, whence the type (Map 1).

BARTLETTINA TEXTITLANA B.L. Turner, sp. nov. Fig. 2

Shrubs to 2.5 m high, minutely pubescent, somewhat viscous. **Leaves** 10-16 cm long, 3-4 cm wide; petioles ca 1 cm long; blades lanceolate, widest near the middle, the juncture of petiole with blades forming a distinct auricle, the margins entire. **Capitulescence** a terminal cymose-panicle ca 6 cm high, 9 cm wide, the ultimate peduncles 4-10 mm long. **Heads** ca 7 mm long, 2 mm wide; involucre bracts 5-6, scarcely imbricate, linear-lanceolate, ca 5 mm long, 1.5 mm wide. **Receptacle** 1 mm across, glabrous,

plane. **Florets** 4 to a head; corollas white, glabrous, 5-6 mm long; lobes 5, ca 1 mm long. **Achenes** glabrous, somewhat viscid, ca 3 mm long; pappus of 30-40 white bristles ca 4 mm long.

TYPE: MEXICO. OAXACA: Distrito, Sola de Vega, Mpio. Santiago Textitlan, “Tierra Blanca,” Bosque de Pino, 2290 m, 16 45 03 N, 97 11 45.9 W, 1 Jan 2007, *Maria Ester Jacob Salinas 1498* (Holotype: MEXU; isotype LL-TEX).

What with its auriculate, short petioles, and few-flowered, scarcely imbricate, heads, this is a very distinct species. Named, in part, from the Municipio, whence the type (Map 1).

Maps for all of the previously described taxa of *Bartlettina* are provided in Turner (2010).

ACKNOWLEDGEMENTS

I am indebted to SERBO for providing plants for identification, and to Jana Kos for editorial assistance.

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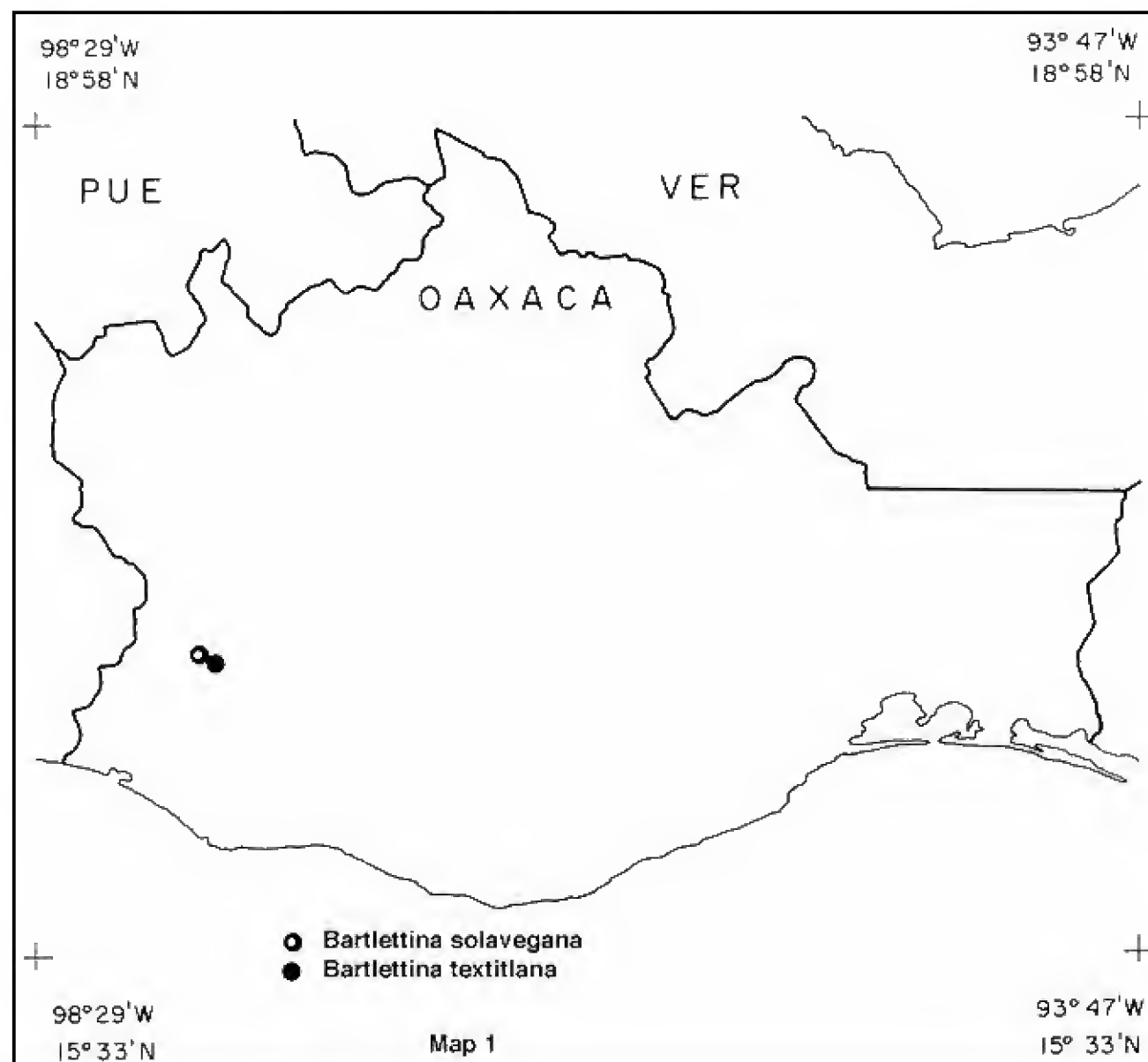




Figure 1. *Bartlettina solavegana* (Holotype).



Figure 2. *Bartlettina textitlana* (Holotype).

The volatile leaf oil of *Juniperus microsperma* and its taxonomy

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ABSTRACT

The composition of the volatile leaf oil of *Juniperus microsperma* is reported and compared with the oils of *J. convallium*, *J. davurica* var. *arenaria*, *J. sabina*, *J. saltuaria* and *J. semiglobosa*. The volatile leaf oil of *J. microsperma* from Song Zong, Xizang is very distinct and is dominated by sabinene (33.9%), pregeijerene B (16.3%), elemol (14.6%) and 8- α -acetoxyelemol (7.1%) with moderate amounts of terpinen-4-ol, germacrene D, and α - and β -eudesmols. There appears to have been such long genetic separation between *J. microsperma* and *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina* that the terpenoids have evolved different patterns due to selection. Thus, the phylogenetic relationships are not discernable in the volatile oil compositions. Published on-line: www.phytologia.org *Phytologia* 95(1): 87-93(Feb. 1, 2013).

KEY WORDS: *Juniperus microsperma*, *J. sabina*, *J. davurica* var. *arenaria*, *J. saltuaria*, *J. semiglobosa*, *J. convallium*, *J. convallium* var. *microsperma*, terpenoids.

The taxonomy of *Juniperus microsperma* (W. C. Cheng & L. K. Fu) R. P. Adams has been unstable. It was originally described as *Sabina convallium* (Rehder & E. H. Wilson) W. C. Cheng & L. K. Fu var. *microsperma* W. C. Cheng & L. K. Fu (*Acta Phytotax. Sin.* 13(4): 86, 1975) then raised to *Sabina convallium* W. C. Cheng & L. K. Fu W. C. Cheng & L. K. Fu (*Fl. Xizangica* 1: 390, 1983). The genus *Sabina* was not recognized so it was changed to *Juniperus convallium* var. *microsperma* (W. C. Cheng & L. K. Fu) Silba (*Phytologia Mem.* 7: 33, 1984). Then more recently raised to the specific level as *Juniperus microsperma* (W. C. Cheng & L. K. Fu) R. P. Adams (*Biochem. Syst. Ecol.* 28: 540, 2000).

Farjon (2005, 2010) recognized *J. convallium* var. *microsperma*, but Adams (2011) recognized *J. microsperma*. Recently, Mao et al (2010) collected materials of *J. microsperma* near the type locality in Song Zong, Xizang (Tibet) and included this collection in a phylogeny of *Juniperus* based on cpDNA sequences. They found that their sample of *J. microsperma* was not related to *J. convallium* (a turbinate cone juniper), but was part of a clade with *J. sabina* and *J. semiglobosa* and junipers from North America (Fig. 1). The multi-seeded *sabina* junipers have seed cones that are oval, ellipsoid, round, reniform or irregular globose with one to several seeds per cone.

The inclusion of *J. microsperma* in *J. convallium* has been problematical because, although some of its seed cones have pointed tips, so that they appear to be turbinate; other cones are oval or globose (Fig. 2). Comparing the seed cones of *J. microsperma*, *J. sabina* var. *arenaria* (now *J. davurica* var. *arenaria*, see Adams and Schwarzbach, 2012), and *J. convallium* (Fig. 3), one can see the similarity in some seed cones. However, the DNA data seems clear that *J. microsperma* is not a member of the turbinate junipers that include *J. convallium*, but rather part of the sabinoid junipers (Fig. 1).

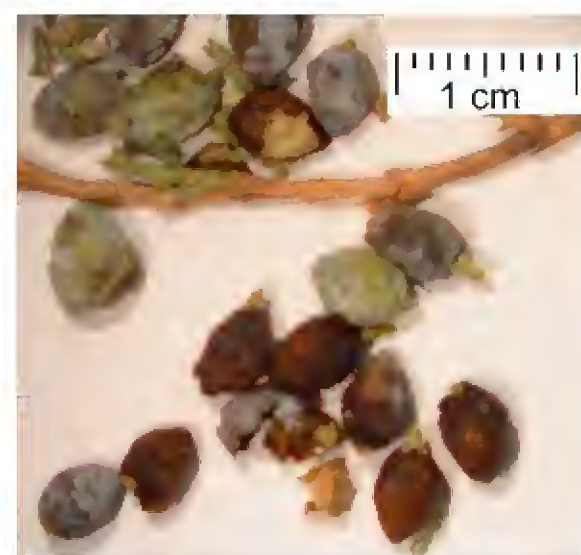
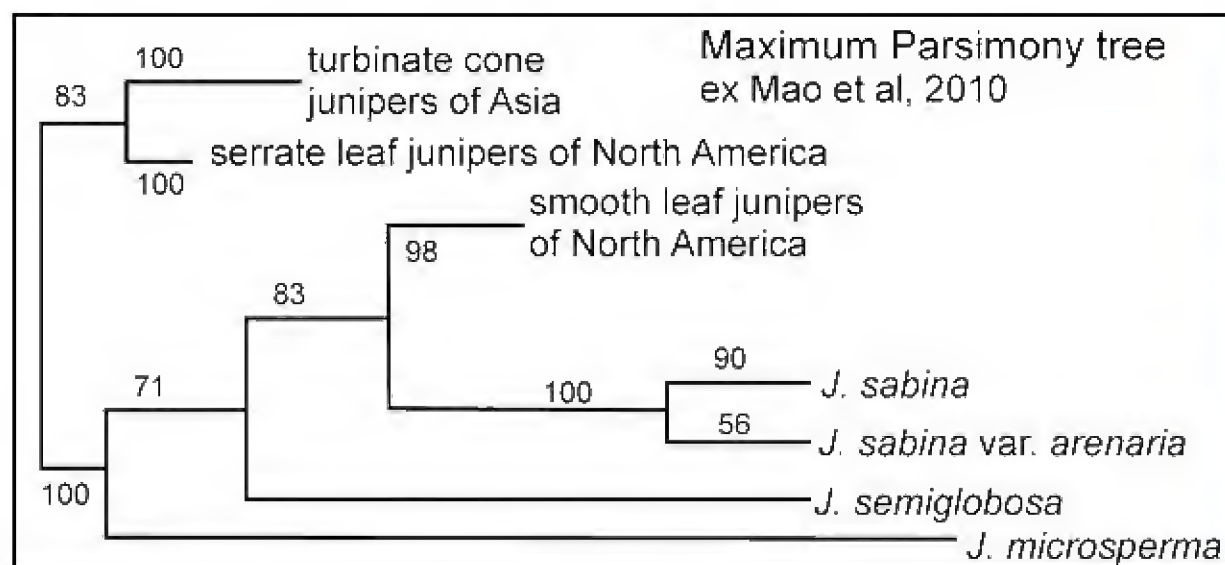


Figure 1. A maximum parsimony tree showing the clade with *J. microsperma*, *J. sabina* and smooth leaf junipers of North America. the numbers at the branch points are bootstrap support values (adapted from Mao et al., 2010).

Fig. 2. Seed cones of *J. microsperma* from J-Q Liu QTP-2011-201 specimen.



Figure 3. left: *J. microsperma*, J-Q Liu 201; center: *J. sabina* var. *arenaria*; right: *J. convallium*.

The purpose of the present paper is to report on the leaf essential oil of *J. microsperma* from Xizang and compare it with its reputed nearest relatives (*J. semiglobosa*, *J. sabina* and *J. davurica* var. *arenaria*, Fig. 1) as well as *J. convallium* and *J. saltuaria* (members of the turbinate junipers). The leaf essential oil of these nearest relatives have been reported: *J. convallium* (Adams, Zhang and Chu, 1993a); *J. davurica* and var. *arenaria* (Adams et al., 1998; Adams, Nguyen and Liu, 2006); *J. saltuaria* (Adams, Zhang and Chu, 1993b), *J. semiglobosa* (Adams et al., 1992; Adams 1999).

MATERIAL AND METHODS

Specimens used in this study (species, location, collection numbers): *J. microsperma*, Song Zong, Xizang (Tibet), China, Jian-Quan Liu QTP-2011-201(lab accession 13633; *J. convallium*, Songpan, Sichuan, China, Adams 8523-8525; *J. davurica* var. *arenaria*, AR, sand dunes, Lake Qinghai, Qinghai, China, Adams 10347-52; *J. sabina*, TS, Tian Shan Mtns., Xinjiang, China, Adams 7836-38; *J. saltuaria*, 23 km se of Forestry Station, Gansu, China, Adams 6788-6790; Deqin Co., Yunnan, China, Adams 8494-96, 8505; *J. semiglobosa*, 60 km sw of Bishket, Kyrgystan, Adams 8210-8212, 2 km s of Dzhabagly, Kazakhstan, Adams 8227, 8229, 8230. Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

Fresh (100 g.) and air dried (10-15 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus with a diethyl ether floating trap (Adams, 1991). The oil samples were

concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

The volatile leaf oil of *J. microsperma* from Song Zong, Xizang is very distinct and dominated by sabinene (33.9%), pregeijerene B (16.3%), elemol (14.6%) and 8- α -acetoxyelemol (7.1%) with moderate amounts of terpinen-4-ol, germacrene D, and α - and β -eudesmols (Table 1). Several compounds that are found in *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina*, such as linalool and 4-epi-abietal, are also found in *J. microsperma* (Table 1). However, several terpenes that seem to typify *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina*, such as cis-piperitol, citronellol, methyl geranate, and germacrene D-4-ol, are not found in *J. microsperma*. In fact, the terpenes of *J. microsperma* seem a little more similar to those of *J. saltuaria* in regards to pregeijerene B, elemol, α - and β -eudesmols, and 8- α -acetoxyelemol (Table 1).

The DNA sequence data is robust in showing *J. microsperma* in a clade with the sabinoid junipers (Fig. 1). Mao et al. (2010) (Table 2, Fig. 4) estimated the branch point where *J. microsperma* unites with *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina* at approximately 19 Mya (10-28 Mya). Although this may not be a long period to accumulate neutrally evolved intergenic and intron mutations, this is a very long time for adaptational changes in defense chemicals (terpenoids) to accumulate. As noted previously (Adams, 2011), the terpenoids provide their most useful taxonomic information at and below the species level. In the present case, there appears to have been such a long genetic separation between *J. microsperma* and *J. davurica* var. *arenaria*, *J. semiglobosa* and *J. sabina* that the terpenoids have evolved different patterns due to selection thus their phylogenetic relationships are not discernable in the volatile oil compositions.

ACKNOWLEDGEMENTS

Thanks to G-L. Chu, China; A. D. Dembitsky, Kazakhstan; A. Lara, Spain; S. Shatar, Mongolia; Y. Turuspekov, Kazakhstan; J-P. Zhong, and J-Q. Liu, China for assistance on field trips. This research was supported in part with funds Baylor University and field collecting funds from National Natural Science Foundation of China, Grant 31100488 to K-S. Mao.

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Table I. Comparisons of the per cent total oil for leaf essential oils for sabin = *J. sabina*, Tian Shan, China; aren = *J. davurica* var. *arenaria*, Qinghai, China; micro = *J. microsperma*, Xizang, China; Gansu; salt = *J. saltuaria*, Yunnan and conv = *J. convallium*. Components that tend to separate the species are highlighted in boldface.

KI	Compound	aren	semi	sabin	micro	salt	conv
921	tricyclene	-	-	-	-	t	0.1
924	α -thujene	0.9	1.5	0.9	0.9	0.9	0.1
932	α-pinene	3.8	2.3	1.9	0.9	4.3	42.9
945	α -fenchene	t	t	t	t	t	t
946	camphene	t	0.1	t	t	t	0.3
969	sabinene	57.1	57.5	46.5	33.9	39.4	1.3
974	β -pinene	t	0.2	t	0.2	0.1	0.8
988	myrcene	3.4	3.1	3.6	0.5	1.2	8.1
996	(methyl 4-methylhexanoate)	0.7	-	-	-	-	-
1001	δ -2-carene	0.2	-	0.1	0.5	0.2	-
1002	α -phellandrene	0.2	0.1	0.1	t	0.1	0.1
1008	δ -3-carene	0.2	t	0.1	-	t	t
1014	α -terpinene	0.9	1.3	0.9	1.0	1.3	0.1
1020	p-cymene	0.1	0.7	0.3	0.3	0.4	1.0
1024	limonene	1.4	0.8	1.3	0.8	0.9	5.3
1025	β -phellandrene	t	0.9	0.9	0.7	0.6	1.2
1036	1,8-cineole	-	-	-	-	-	t
1036	2-heptyl acetate	-	-	-	-	-	t
1044	(E)- β -ocimene	0.2	0.2	0.2	-	-	-
1054	γ -terpinene	1.5	2.0	1.4	1.8	2.2	0.2
1065	cis-sabinene hydrate	1.5	0.7	0.7	0.7	0.7	0.2
1074	trans-linalool oxide(furanoid)	-	-	-	-	-	t
1086	terpinolene	0.9	0.9	0.9	0.6	0.8	1.0
1087	2-nonanone	0.5	-	-	-	-	-
1095	trans-sabinene hydrate	0.9	0.6	0.3	0.5	0.8	0.1
1095	linalool	0.8	0.5	0.8	0.4	-	0.7
1100	nonanal	t	-	t	-	-	-
1101	cis-thujone (= α -thujone)	-	-	t	-	-	-
1102	isoamyl-isovalerate	0.2	-	-	-	-	-
1106	cis-rose oxide	-	t	-	-	-	-
1112	trans-thujone(= β -thujone)	-	0.1	0.3	-	-	-
1116	3-methyl-3-butenyl-isovalerate	0.5	-	-	0.1	0.1	-
1118	cis-p-menth-2-en-1-ol	0.3	0.3	0.2	0.3	0.3	0.2
1122	α -campholenal	-	-	-	-	-	0.1
1137	trans-sabinol	-	-	0.2	-	-	t
1137	trans-p-menth-2-en-1-ol	0.2	0.2	0.2	0.2	0.2	0.3
1137	trans-verbenol	-	-	-	-	-	0.3
1147	3-methyl-2-butenyl-3-methyl butanoate	-	-	-	0.1	-	-
1148	citronellal	0.3	0.2	t	-	-	-
1156	sabina ketone	-	0.1	-	-	-	-
1165	2-allyl-phenol, isomer	-	0.1	-	-	-	-
1165	borneol	-	-	-	0.3	-	-
1174	terpinen-4-ol	3.2	4.4	3.0	2.0	4.9	0.3
1179	p-cymen-8-ol	-	t	-	0.1	t	-
1186	α -terpineol	0.1	0.2	0.2	t	0.2	t
1193	(Z)-4-decenal	0.1	-	t	-	-	-

KI	Compound	aren	semi	sabin	micro	salt	conv
1195	cis-piperitol	0.2	0.2	0.1	-	0.1	-
1204	verbenone	-	-	-	-	-	0.2
1207	trans-piperitol	t	0.1	0.1	0.1	0.1	-
1219	coahuilensol, methyl ether	-	0.1	-	-	-	-
1223	citronellol	1.7	0.7	0.1	-	-	-
1232	(3Z)-hexenyl-3-methyl butanoate	-	-	-	0.6	t	-
1241	carvacrol, methyl ether	-	-	-	-	t	-
1249	piperitone	0.2	-	0.1	t	0.1	0.2
1255	(Z)-4-decen-1-ol	-	t	t	-	-	-
1257	linalyl acetate	0.3	-	0.4	-	-	-
1257	methyl citronellate	2.0	1.1	0.2	-	-	-
1274	pregeijerene B	-	-	-	16.3	3.1	-
1285	bornyl acetate	t	0.2	0.1	0.2	0.1	1.1
1287	trans-linalool oxide acetate	-	-	t	-	-	-
1290	trans-sabinyl acetate	2.6	-	15.9	-	-	-
1293	2-undecanone	1.2	0.2	-	-	-	0.3
1298	carvacrol	-	-	-	-	0.1	0.1
1315	(E,E)-2,4-decadienal	t	0.2	-	-	-	-
1320	aromatic phenolic,<u>149</u>,91,134,164	-	-	-	-	0.3	1.0
1322	methyl geranate	0.7	0.4	0.5	-	-	-
1345	α -cubebene	-	-	-	-	-	0.6
1346	α -terpinyl acetate	t	-	0.3	-	-	-
1350	citronellyl acetate	0.4	-	-	-	-	-
1374	α -copaene	-	-	-	-	-	0.1
1379	geranyl acetate	0.3	-	-	-	-	-
1387	β -bourbonene	-	-	-	0.1	-	-
1387	β -cubebene	-	-	-	-	-	1.0
1389	β -elemene	-	-	-	0.2	-	-
1410	α -cedrene	-	0.4	0.3	-	0.1	-
1417	(E)-caryophyllene(β-caryophyllene)	-	-	-	0.8	t	0.2
1419	β -cedrene	-	0.3	0.2	-	-	-
1429	cis-thujopsene	-	0.2	0.2	-	-	-
1448	cis-muurolo-3,5-diene	-	-	-	-	-	1.3
1448	sesquiterpene, <u>43</u> , 105, 147, 220	-	-	-	0.8	-	-
1452	α -humulene	-	-	-	t	t	0.2
1469	n-dodecanol	-	-	-	-	0.6	-
1475	trans-cadina-1(6),4-diene	-	-	-	-	-	1.1
1478	γ -muurolene	0.1	-	-	-	-	-
1480	germacrene D	0.1	-	-	2.4	-	0.5
1489	δ -selinene	-	-	-	t	-	-
1493	trans-murrola-4(14),5-diene	-	-	-	-	-	3.3
1493	epi-cubebol	0.1	-	-	-	-	1.2
1495	γ -amorphene	-	t	-	-	-	-
1500	α -muurolene	0.2	-	0.1	t	0.1	0.4
1513	γ -cadinene	0.6	t	0.2	t	0.2	-
1513	cubebol	-	-	-	-	-	4.7
1518	endo-1-bourbonol	0.1	-	-	-	-	-
1522	δ -cadinene	0.8	0.2	0.2	t	0.4	2.1
1528	zonarene	-	-	-	-	-	0.6
1533	trans-cadina-1,4-diene	-	-	t	-	-	0.5
1537	α -cadinene	0.1	-	t	-	-	-
1539	α -copaen-11-ol	-	-	-	0.3	-	-

KI	Compound	aren	semi	sabin	micro	salt	conv
1548	elemol	t	0.1	0.1	14.6	3.8	-
1559	germacrene B	t	0.1	-	0.2	0.1	-
1561	(E)-nerolidol	-	-	t	-	-	-
1565	(Z)-3-hexenyl benzoate	-	-	-	0.2	-	-
1574	germacrene D-4-ol	3.5	0.3	0.6	-	0.5	0.4
1587	trans-murrol-5-en-4- α -ol	-	-	-	-	-	0.6
1587	allo-cedrol	-	0.7	0.6	-	-	-
1600	cedrol	-	14.3	13.2	-	1.6	0.1
1607	β -oplophenone	0.3	t	0.1	-	-	t
1608	humulene epoxide II	-	-	-	-	-	0.1
1627	1-epi-cubenol	t	-	0.1	-	-	2.5
1630	γ -eudesmol	-	-	-	0.8	0.7	-
1632	α -acorenol	-	-	t	-	-	-
1638	epi- α -cadinol	0.4	t	0.1	0.2	0.2	t
1640	epi- α -muurolol	0.4	t	0.1	0.2	0.3	-
1644	α -muurolol	0.1	-	t	-	-	-
1645	cubenol	-	-	-	-	-	0.6
1649	β-eudesmol	-	-	-	1.5	0.7	-
1652	α-eudesmol	-	-	-	1.4	0.8	-
1652	α -cadinol	1.0	0.2	0.4	-	0.4	0.4
1670	bulnesol	-	-	t	0.5	0.4	-
1671	n-tetradecanol	-	-	-	-	0.3	-
1688	shyobunol	0.1	0.2	0.1	-	-	-
1740	(E,E)-2,6-farnesol	t	-	-	-	-	-
1792	8-α-acetoxyelemol	-	-	-	7.1	2.4	-
1901	epi-laurenene	-	-	-	-	-	-
1905	iso-pimara-9(11),15-diene	-	-	-	-	-	-
1907	pimara-8(11),15-diene	-	-	-	-	0.7	-
1958	iso-pimara-8(14),15-diene	-	-	-	-	1.0	0.2
1978	manoyl oxide	-	t	-	-	-	-
1987	iso-pimara-7,15-diene	-	-	-	-	0.1	1.1
2055	abietatriene	t	-	t	t	1.6	3.5
2056	manool	-	-	-	-	14.8	-
2087	abietadiene	0.1	-	t	t	0.5	3.0
2132	nezukol	-	-	-	-	1.3	-
2282	sempervirol	-	-	-	-	0.3	0.2
2298	4-epi-abietal	0.4	0.1	t	0.3	-	-
2312	abieta-7,13-dien-3-one	1.2	-	0.1	-	-	-
2314	trans-totarol	-	-	-	-	0.7	0.6
2331	trans-ferruginol	t	-	-	-	t	t
2402	abietol	0.1	-	-	-	-	-

KI = Kovat's Retention Index on DB-5(=SE54) column using alkanes. Compounds in parenthesis () are tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Taxonomy of Douglas fir (*Pseudotsuga menziesii*) infraspecific taxa: vars. *menziesii*, *glauca* and *oaxacana*: nrDNA, cpDNA sequences and leaf essential oils

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ABSTRACT

Six chloroplast DNA regions of Douglas fir (*Pseudotsuga menziesii*) from trees in 11 populations from Washington southward to Oaxaca, Mexico were sequenced yielding 4857 bp of data as well as sequences for nrDNA from *P. macrocarpa*, *P. menziesii* var. *menziesii*, (3 populations) and var. *glauca* (partial, NM population). *Pseudotsuga macrocarpa* was included as an outgroup. The nrDNA grouped the three var. *menziesii* separate from var. *glauca* (NM), even with only partial sequences. Very little variation was found among the populations of *P. menziesii* but the cpDNA did give support for the recognition of var. *menziesii* and var. *glauca*, with some support for var. *oaxacana*. However, for the cpDNA data, the population of *P. m.* var. *menziesii* on serpentine soil in sw Oregon had its highest affinity to var. *glauca* in Arizona. In contrast, a previous study using terpenoids (Adams et al. 2012) clearly placed the serpentine soil Oregon trees with var. *menziesii*. The Oregon population may be introgressed by the chloroplast from inland (var. *glauca*) germplasm, leading to these results. The var. *oaxacana* differed by 3 MEs (mutational events) from var. *glauca*, and by 2 MEs from a tree in nearby El Chico NP, Hgo. *Pseudotsuga menziesii* var. *glauca* appears very uniform for these cpDNA markers from Wyoming to Arizona, New Mexico, and into northern Mexico. The terpenoid data seem to reflect the status of current evolution and the cpDNA data indicate ancestral relationships.

Published on-line: www.phytologia.org *Phytologia* 95(1):94-102 (Feb. 1 2013).

KEY WORDS: *Pseudotsuga menziesii*, var. *menziesii*, var. *glauca*, var. *oaxacana*, taxonomy, nrDNA, cpDNA sequences, Douglas fir, *Pseudotsuga macrocarpa*.

Douglas fir [*Pseudotsuga menziesii* (Mirb.) Franco] is a wide-ranging, common forest tree in North America (Fig. 1). The nomenclatural history of the name is a morass, but seems to have been settled by James Reveal (see <http://www.plantsystematics.org/reveal/pbio/LnC/dougfir.html>).

In a recent treatment, Eckenwalder (p. 572, 2009) recognizes two varieties: var. *menziesii* and var. *glauca* (Mayr) Franco [cited as (Beissn.) Franco in Eckenwalder, 2009]. Eckenwalder (2009) did not recognize var. *oaxacana* Debreczy & Racz, described from Oaxaca (Debreczy and Racz, 1995).

The leaf essential oils of *P. menziesii* have been exhaustively studied by von Rudloff (1972, 1973, 1984) and von Rudloff and Rehfeldt (1980) who carefully documented the large differences in oil composition between coastal (var. *menziesii*) and inland (var. *glauca*) varieties.

A second team from USDA, Forest Products, Richmond, CA (Snajberk and Zavarin), conducted extensive studies of the terpenoids from the oleoresin of Douglas fir (Snajberk, Lee and Zavarin, 1974; Snajberk and Zavarin, 1976; Zavarin and Snajberk, 1973, 1975). In their most comprehensive study (Snajberk and Zavarin, 1976), they found four chemical races: coastal, northern inland, southern inland and Sierra Nevada. These are shown in Fig. 1, along with populations used in the present study.

There has been previous work at the molecular level on Douglas fir in Mexico. Li and Adams (1989) reported that allozymes divided the Douglas fir into northern coastal (var. *menziesii*) and inland (var. *glauca*) groups, and further divided the inland group into two subgroups (northern and southern inland). They did not find evidence of a subgroup of Sierra Nevada Douglas fir, as Snajberk and Zavarin (1976) found, this based on the oleoresin oils. In addition, Li and Adams (1989) found a distinct pattern in the allozymes from population 103 at General Cepeda, Coah., MX and speculated that it might be *P. flahaultii* Flous (also recognized by Martinez, 1963). However, a nearby collection (104, La Encantada, near Zaragoza, NL) clustered closely with *P. menziesii* from New Mexico, making this an unresolved case.

Gugger et al. (2010) examined mtDNA and cpDNA sequences and found support for coastal (var. *menziesii*) and inland (var. *glauca*) divisions in the United States and Canada. No evidence was found for a Sierra Nevada taxon, but mtDNA suggested the inland (var. *glauca*) taxon might be divided into northern and southern groups. In a subsequent study, Gugger et al. (2011) examined Douglas fir from Mexico. They found considerable divergence in cpDNA from Cerro Potosi, NL and Jamé, Coah. and from other Mexico populations. CpSSRs supported two clades in Mexico (Gugger, Fig. 4c), but that pattern was not recovered with mtDNA (Gugger, Fig. 4a) or cpDNA (Gugger, Fig. 4b) data. In summary, they concluded that "Mexican populations were genetically distinct from USA and Canadian populations, but more closely related to the Rocky Mountain variety than the coastal variety". As Gugger et al. (2010) did not show data from Mexico, and Gugger et al. (2011) showed only data from that country, it is difficult to ascertain the relationship of Mexican populations to those of the USA.

Phenotypic analyses (Reyes Hernández et al. 2006) revealed that *Pseudotsuga* populations of northern Mexico are morphologically similar to *P. menziesii* var. *glauca* from southwestern USA, but the populations from central Mexico differed. They also found a population of NE Mexico (San Francisco) morphologically separated from the rest, even from those of the same geographical region, suggesting an effect of microhabitat selection. This population is just 15 km NW from the one from Cerro Potosi analyzed here.

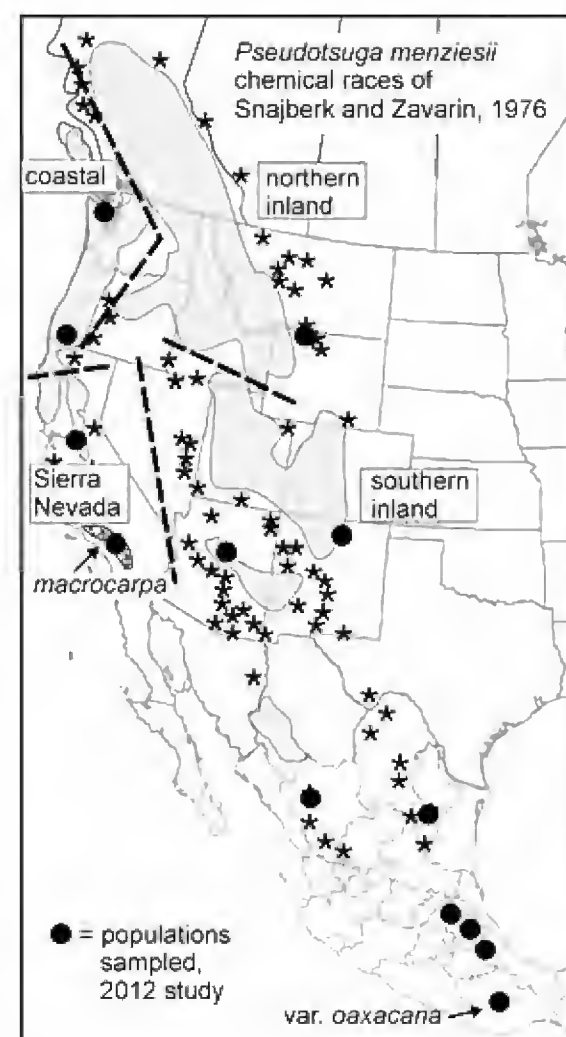


Figure 1. Distribution of *P. menziesii* with chemical races (dashed lines) of Snajberk and Zavarin (1976). Stars denote outlying locations.

The leaf terpenes of *Pseudotsuga menziesii* were analyzed by Adams et al. (2012) from throughout its range (Fig. 1). They found (Fig. 2) that terpenes separated Douglas fir into the two classical varieties: var. *menziesii* (coastal) and var. *glauca* (inland). In addition, there appeared to be a slight differentiation between the Yellowstone, AZ-NM and central Mexico populations (Fig. 2). The Cerro Potosi leaf oils also showed some local differentiation (Fig. 2). All three of the populations of *P.*

menziesii var. *menziesii* from the coast were very similar in their oils (Fig. 2). In addition, they reported a north-south cline from Wyoming to southern Mexico but found little support for var. *oaxacana* (Fig. 3).

The present study was undertaken to complement the terpenoid study of Adams et al. 2012) by sampling the same populations and analyzing six cpDNA regions from the USA and Mexico.

MATERIALS AND METHODS

Plant material (Fig. 4): *P. menziesii* var. *menziesii* (coastal/ Sierra Nevada): Adams 13239-13240, Olympic National Park, 48° 02' 48.1"N, 123° 25' 04.08"W. elev. 1720 ft, Adams 12745-12757, on serpentine soil, Oregon Mtn., OR, 41° 59' 59.1" N, 123° 47' 10.2" W, 895 m; Adams 12779-12783, 6 km e of Buck Meadows, CA, 21 km w of Yosemite NP on US 120, 37° 49.579' N, 119° 58.421' W, 1150 m. var. *glauca*: Adams 12556-12560, 13 km w of Cimarron, NM on US 64, 36.54684° N, 105.03321° W, 2125 m; Adams 12744-12748 (ex D. Thornburg, 1-5), 9 km ne of Pine, AZ on Hwy 87, 34° 27.422' N, 111° 24.115' W, 2250 m; Adams 12818-12822, 20 km e of Yellowstone NP, on US 14 at the Palisades, 44.45448° N, 109.78182° W, 1910 m; Adams 13056-13060, (ex M. Socorro González Elizondo 7777a-e), Cerro Potosi, NL, 24° 53' 9" N, 100° 13' 14" W, 3141 m.; Adams 13236-13238 (ex Marie Deslauriers, Quebec), Cerro Catana, Coah., Adams 13061-13066, (ex Martha González Elizondo 4408-4409, 4413-4416) Los Altares, Dur., 25°2'56" N, 105°59'48" W, 2310 m; Adams 13082-13087 (ex Vargas-Hernandez J1-J6), El Chico Natl. Park, Mineral del Chico, Hgo., 20° 10' 16" N, 98° 43' 55" W, 2,765 m, var. *oaxacana*: Adams 13101-13103, 13105-13106 (ex Vargas-Hernandez II-16, Paraje Peña Prieta, Oaxaca, 17° 09' 38" N, 96° 38' 07" W, 2,700 m.

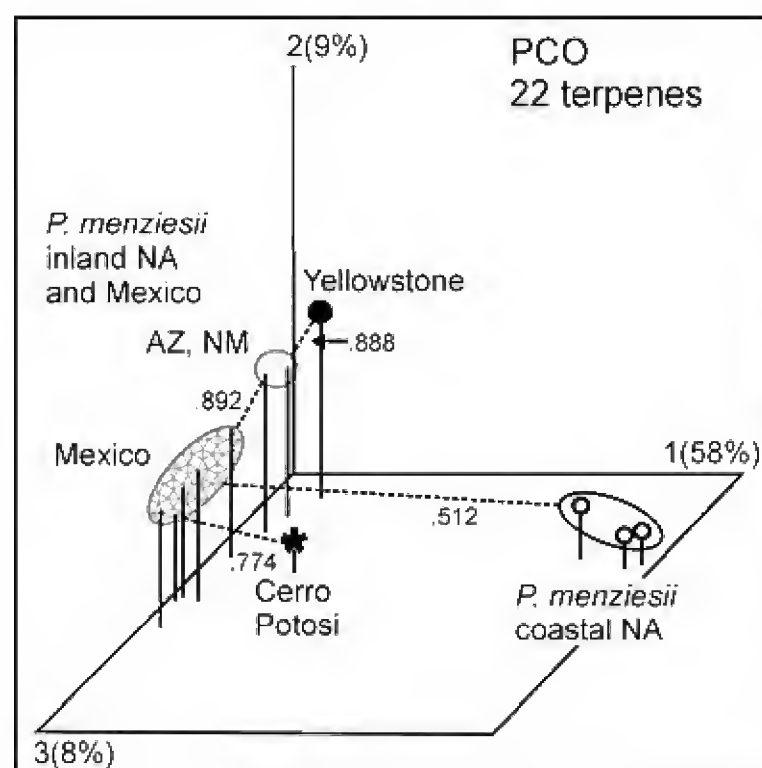


Figure 2. PCO based on 22 terpenes (from Adams et al. 2012).

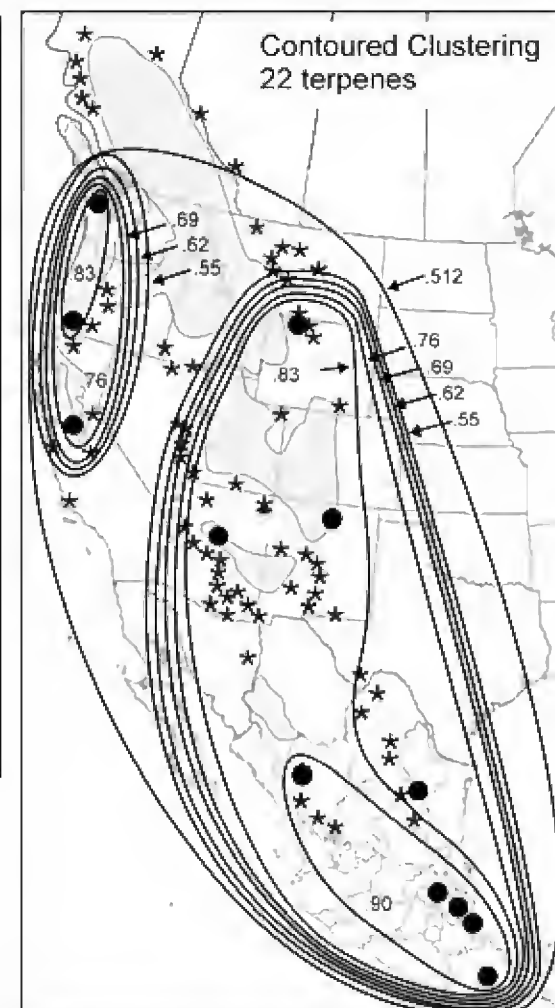


Figure 3. Contoured clustering (from Adams et al., 2012)

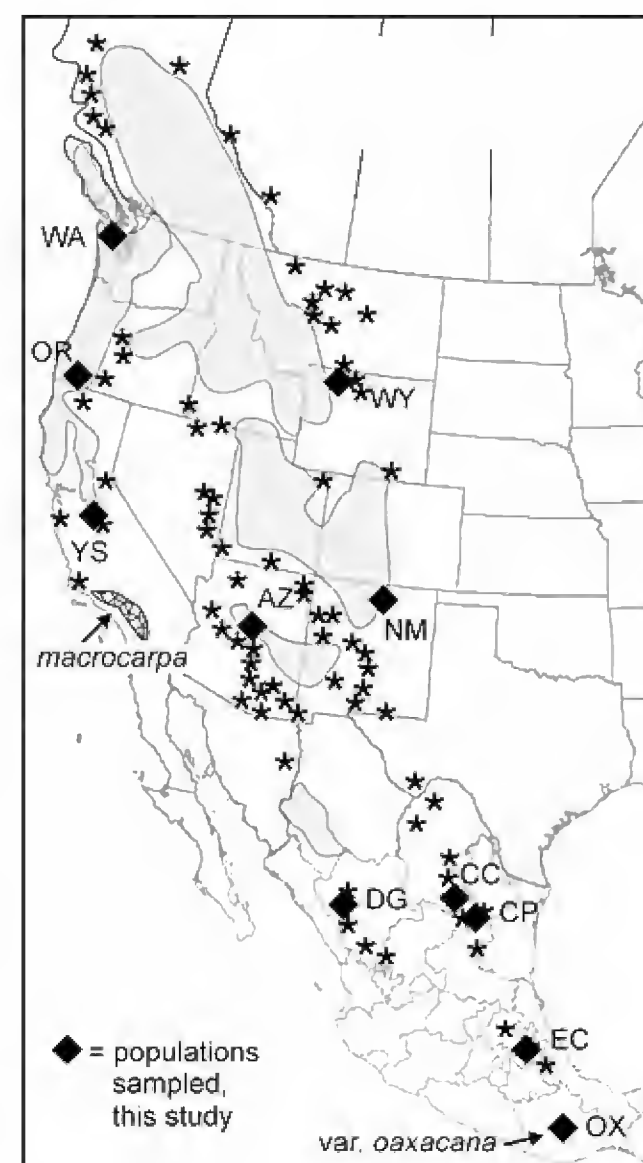


Figure 4. Populations sampled in this study, including *P. macrocarpa*.

P. macrocarpa: Adams 12776-12778, USFS Eddy Arboretum, Placerville, CA. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU, CIIDIR and CHAPA).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

PCR amplification: Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. nrDNA was amplified by use of standard ITSA, ITSB, ITS739F, ITS739R, CAA123F primers (Adams et al. 2011).

Chloroplast primers were synthesized based on GenBank sequences for *Pseudotsuga menziesii* and *P. macrocarpa* for the following cp regions (name, forward and reverse primer, Tm):

psbA-matK: psbA57f	GTTTTTCGGTGCTAGTAATC	matK16r	CAGGATCTGAAAGTAGAAAA, 50°C
trnM-trnS: trnM30f	AAGGCTCATAACCTTGAG	trnS1006r	TACTATACCGGTTTTCAAGA, 50°C
psaJ-petG: psaJ8f	TGGAAAGATAGGTCTTTAGAT	petG27r	AACTGCATATTCACAATACC, 50°C
rbcL-atpB: rbcL20f	AATCCGACACTAGCTTTAG	atpB724r	AATACGTCCCACATTTTTT 50°C
petN-rpoB: petN25f	AAAAACTACCATTAAAGCAG	rpoB6r	CTCCCTCATTTTCATCTAAT 50°C
trnS-clpP: trnS32f	CGTACTACGGATTAGCAA	clpP11r	AGGAACTTTTGGGACAC 50°C

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.). Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

The nrDNA proved recalcitrant in sequencing for var. *glauca* and all the materials from Mexico. Repeated efforts and the synthesis of gene-walking primers failed to generate complete sequences. Complete sequences for all the var. *menziesii* and *P. macrocarpa* plants were obtained, but only var. *glauca* from New Mexico (NM) yielded partial sequences. Analysis revealed one (1) substitution and two (2) indel differences between var. *menziesii* (WA, OR, YS) and var. *glauca* (NM). A Neighbor Joining tree (NJ) showed high support for the vars. *menziesii* and *glauca*, even with partial sequences from the NM plants.

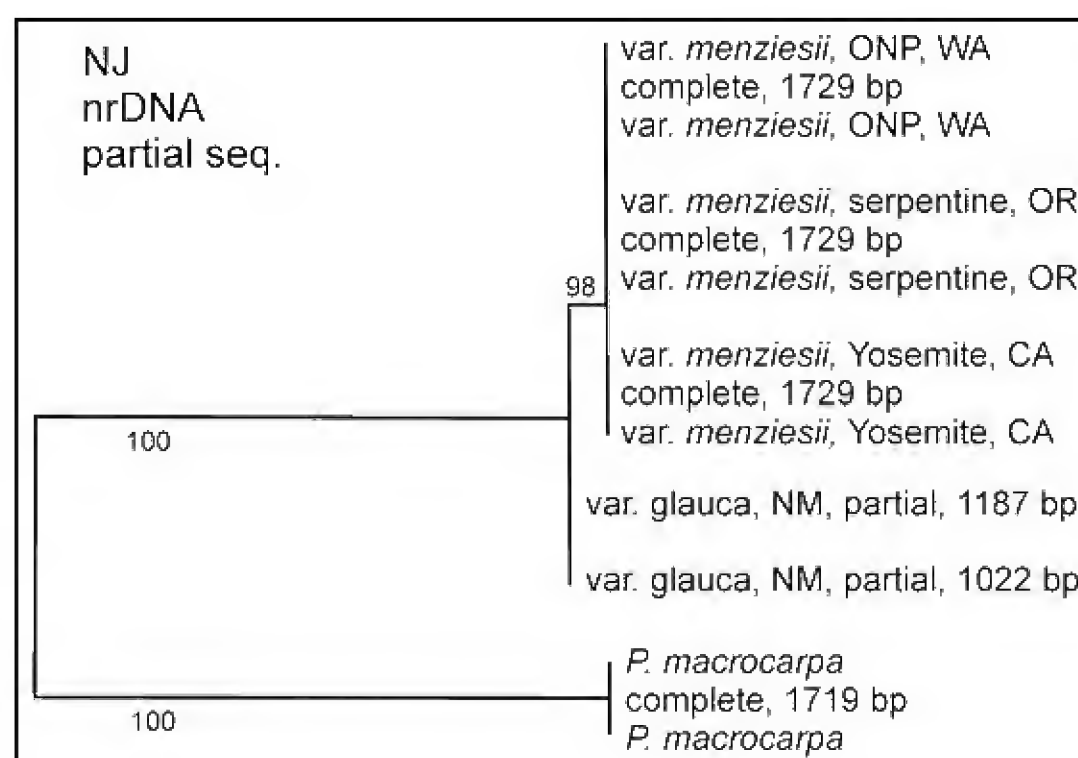


Figure 5. NJ analysis based on nrDNA. Note the partial sequences for the NM plants (1187, 1022 bp).

Sequencing the six cpDNA regions resulted in few mutations (Table 1). Most substitutions were either between *P. macrocarpa* and *P. menziesii*, or mutational events that were only found once.

Table 1 Chloroplast DNA regions sequenced in Douglas fir. subs = substitution event.

cp region	length(bp)	# subs.	# indels	# mut. events (ME)	comments
psbA-matK	814	6	1	7	6 subs. in <i>P. macrocarpa</i>
trnM-trnG-psbZ-trnS	932	11	3	14	3 subs. in <i>P. macrocarpa</i>
psaJ-petG	781	3	1	4	
rbcL-atpB	731	3	1	4	
petN-rpoB	795	3	3	6	2 subs. in <i>P. macrocarpa</i>
trnS-clpP	804	6	2	8	2 subs. in <i>P. macrocarpa</i>
totals	4857	32	11	43	

The most variable region was trnM-trnG-psbZ-trnS with 14 MEs (Table 1) and psaJ-petG and rbcL-atpB were the least variable regions with only 4 MEs. Of the 32 substitutions, only 13 were found multiple times in *P. menziesii*. Of the 11 indels, only 2 were present multiple times in *P. menziesii*.

A Neighbor-Joining (NJ) analysis resulted in a tree with very low boot strap values for most branches (Fig. 6). There is support for var. *menziesii* (59) and var. *oaxacana* (56) and limited support for var. *glauca*. This is in strong contrast to the terpenoid data (Fig. 2). The population on serpentine soil in Oregon (OR, Fig. 3) is not in the clade with var. *menziesii* from Olympic NP and Yosemite NP (Fig. 6). The two plants from Yellowstone, WY are somewhat separated (Fig. 6).

However, it should be noted that several studies have shown that var. *menziesii* and var. *glauca* hybridize and form introgressants inland from the coast to the rocky mountains (von Rudloff 1972; 1973; 1984; von Rudloff and Rehfeldt 1980). It is very likely that our samples may contain some introgressed individuals. If so, then a phylogenetic algorithm is not the best choice for analysis of this data set.

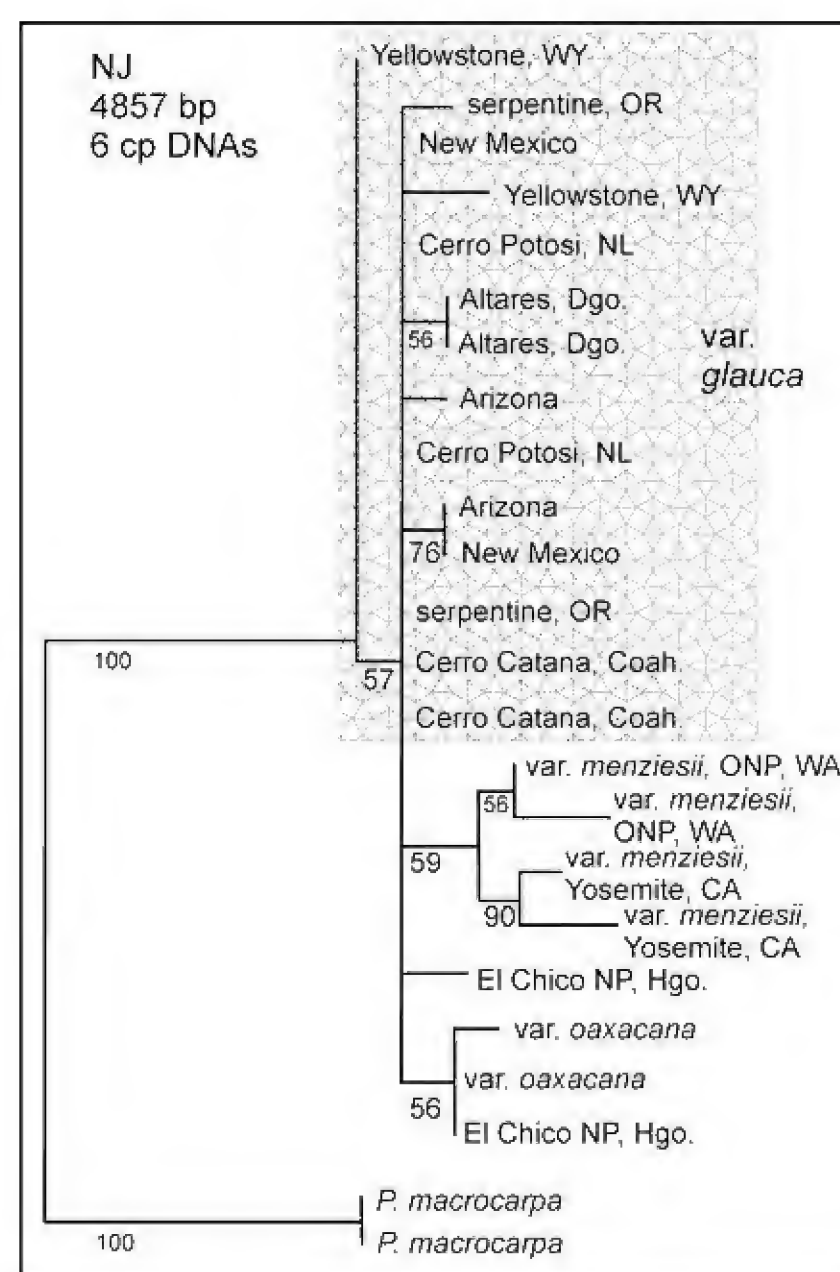


Figure 6. NJ based on 4857 bp of cpDNA data.

A UPGMA analysis of the data revealed a slightly different pattern (Fig. 7) in which vars. *menziesii*, *glauca* and *oaxacana* are resolved into groups. Again, the serpentine soil, OR plants are grouped with var. *glauca*. Interestingly, var. *oaxacana* is now grouped with the other Mexican populations, except for the Cerro Catana plants (CC, Fig. 3) that are grouped with the inland plants of AZ, NM and WY (Fig. 7).

Additional insight can be obtained by examination of the mutations in a minimum spanning network (MSN). This analysis involved only the 15 mutations that occurred more than once in the individuals of *P. menziesii*. Of these 15 MEs, 13 were substitutions and 2 were indels. The MSN shows that by DNA data var. *menziesii* consists of only the Olympic NP and Yosemite, CA plants (Fig. 8) and within the Olympic NP plants (WA), there are 3 MEs. That group is separated by only 4 MEs from var. *glauca* / var. *oaxacana*. In short, it is clearly not well defined by cpDNA data. The entire var. *glauca* group from the Rocky Mountains to northern Mexico differs among populations by only 0, 1 or 2 MEs (Fig. 8). There is a hint of divergence in the central Mexico populations of Durango and Cerro Catana, as they differ by 2 MEs, and the group differs from other var. *glauca* by only 2 MEs (Fig. 8). The var. *oaxacana* group is separated by 3 MEs, but in itself, is diverse, having 2 MEs differences compared to a plant from El Chico, which differs by 3 MEs from another El Chico plant (Fig. 8).

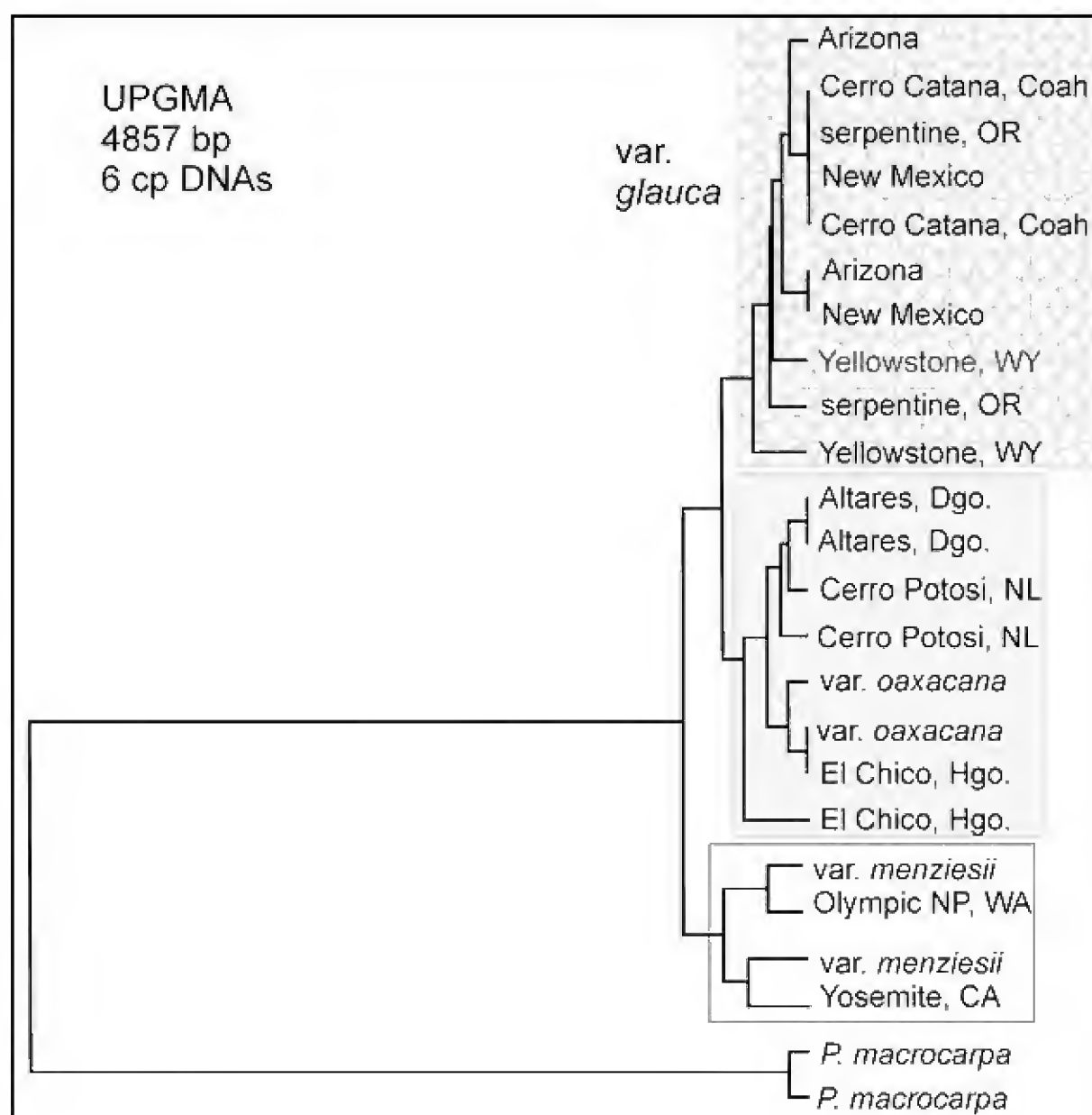


Figure 7. UPGMA based on 4857 bp from 6 cpDNAs

Mapping the MSN onto a map gives a geographic perspective (Fig. 9). Very few mutations separate the populations of Douglas fir from OR, WY, AZ, NM, CC and CP. The Douglas fir on serpentine soil (southern Oregon, OR) differs by only 1 ME from Arizona (var. *glauca*, Fig. 8), however, its terpenes were like those of coastal Douglas fir (var. *menziesii*, Fig. 2 above). It may be that the Oregon population has been introgressed by inland Douglas fir (var. *glauca*).

There were no differences between Arizona (AZ) Douglas fir and Cerro Potosi (CP, Fig. 9); and only one ME difference was found between AZ and CC (Cerro Catana, Fig. 9). Clearly, the cpDNA data show very few changes in sequences in these intergenic regions from Wyoming southward to northern Mexico.

The Altares, Dgo. (DG) trees differ by 2 MEs from Cerro Potosi populations (CP, Fig. 9), indicating they are part of the var. *glauca* complex (at least in these cpDNA data).

The results from 6 cpDNAs are similar to those of Wei et al. (Fig. 1, 2011) who, using only trnM-trnS data, found haplotypes characteristic of var. *menziesii* (coastal, Pacific Northwest), var. *glauca* (inland, northern Rockies to southern New Mexico) and var. *oaxacana* (central Mexico to Oaxaca). Using data from the first intron of nad7 (mt DNA), they found haplotypes supporting var. *menziesii* (coastal, Pacific Northwest) and var. *glauca* (inland, northern Rockies to southern Mexico), with no support for var. *oaxacana*. It is of interest that they date the split of var. *menziesii* from *glauca* as 8.5 Ma

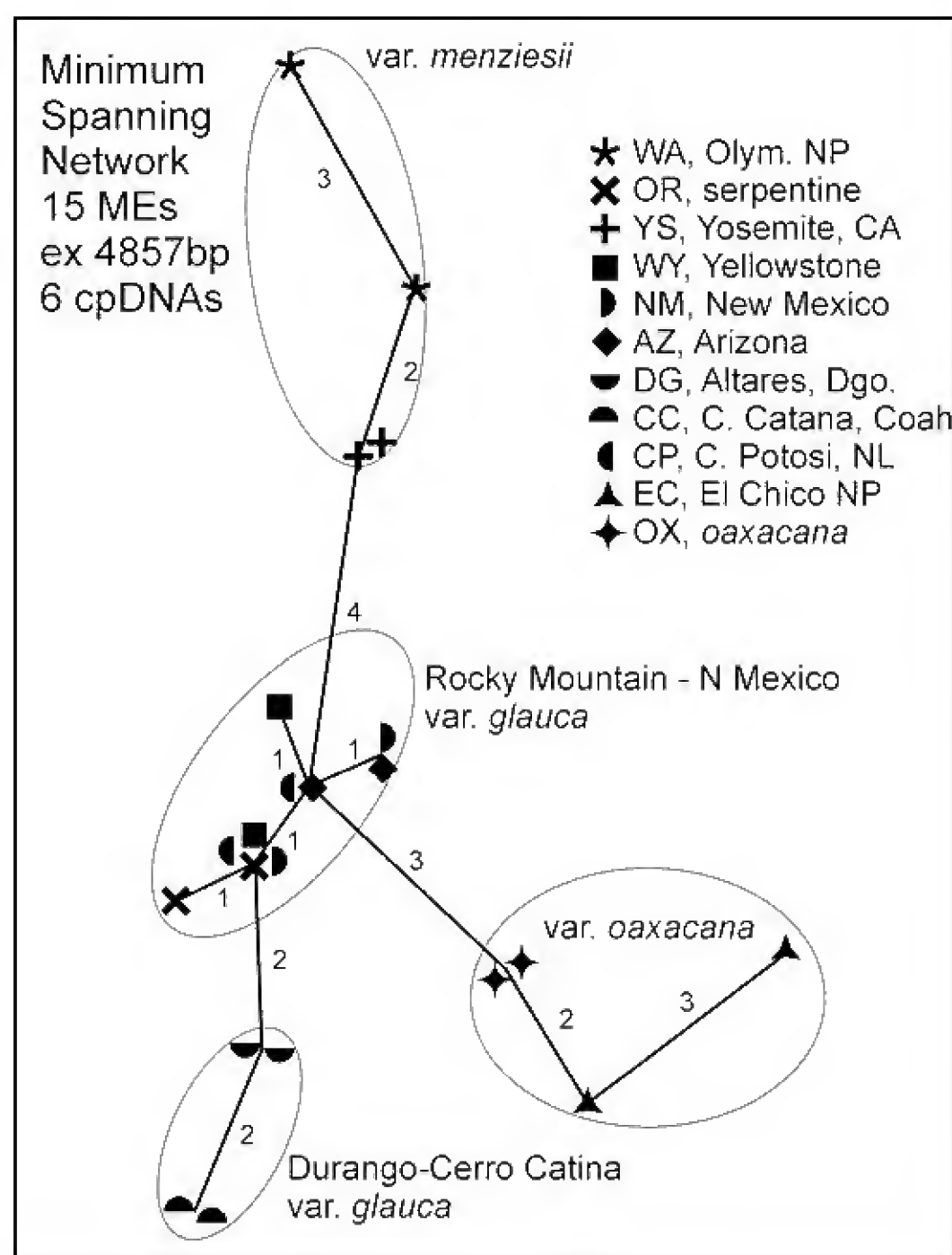


Figure 8. Minimum spanning network based on 15 MEs.

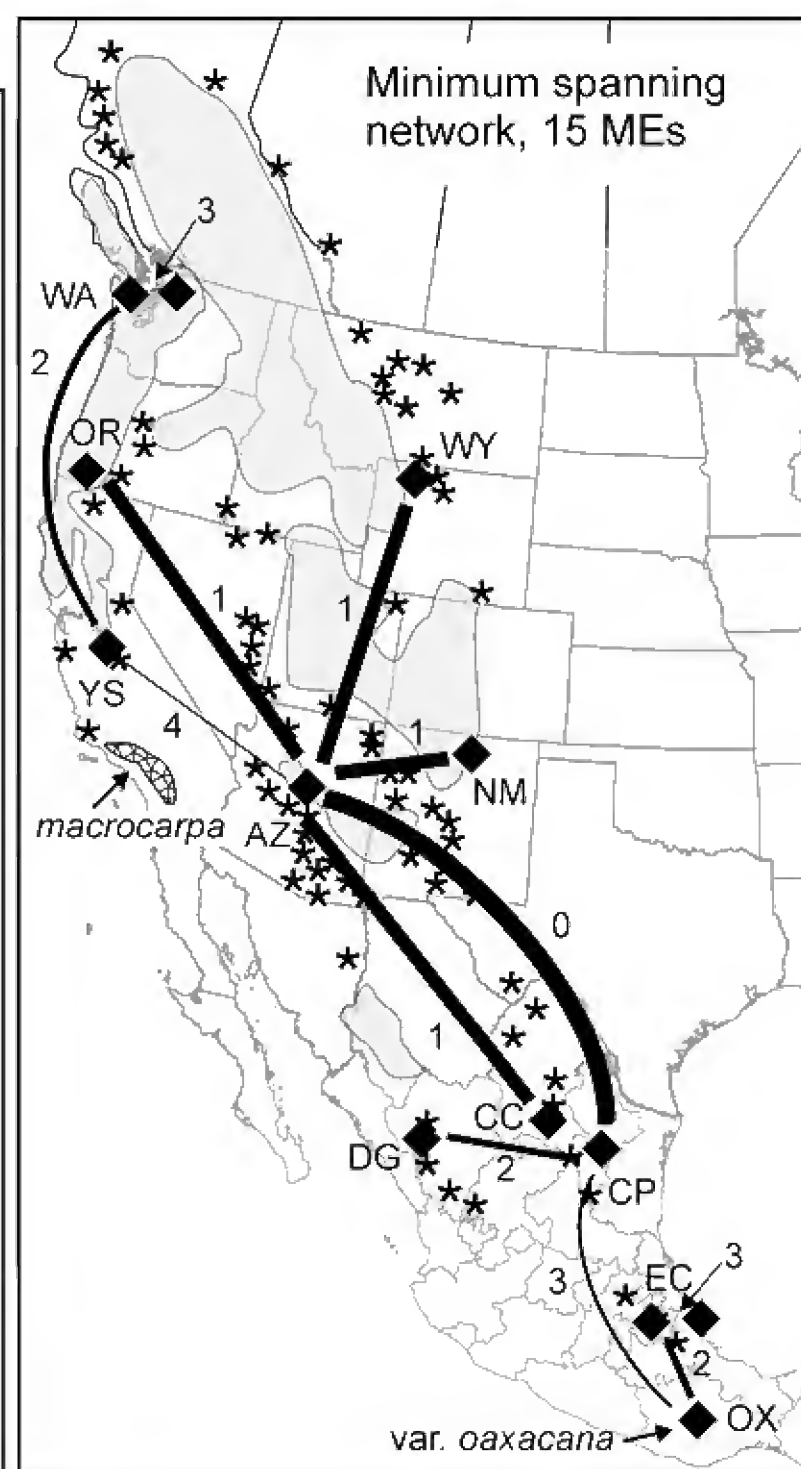


Figure 9. MSN on geographic map. Numbers next to lines are # MEs. Line width is inversely proportional to # ME differences.

and divergence of the Mexican populations as 3.2 - 4.8 Ma. Syring et al. (2007) concluded that the presence of shared haplotypes among *Pinus* species was due to incomplete lineage sorting. They estimated that reciprocal monophyly will be more likely than paraphyly in 1.7 to 2.4 Ma with complete genome-wide coalescence in species in up to 76 Ma. If Wei et al. (2011) are correct about the ages of the divergence of *Pseudotsuga* varieties (3.2 to 8.5 Ma), and Syring et al. (2007) are correct in their dates, then it is not unexpected that one would find ancestral haplotypes from one variety in the genome of another variety. This could explain our finding of var. *glauca* haplotypes in the southern Oregon population. Wei et al. (2011) did not find var. *glauca* haplotypes in their southern Oregon population, but they did find var. *glauca* haplotypes in their central Oregon population (Wei et al. (Fig. 1, 2011). Their trnM-trnS data showed a similar result.

The difference in the terpene data, the nrDNA and the cpDNA data for the southern Oregon, serpentine population is a major difference in the two studies. The terpenes from the Oregon population are most similar to var. *menziesii* from Olympic NP, WA (Fig. 2 above and Adams et al. 2012). Adams and Stoeckl (2012) showed that the terpenes from hybrids between var. *menziesii* (coastal) and var. *glauca* (inland) are more like var. *glauca* (inland) than var. *menziesii*, due to dominant inheritance towards var.

glauca. If the southern Oregon population is of hybrid origin (var. *menziesii* x var. *glauca*), then one might expect the terpenes to be like var. *glauca*. But this is not the case. The terpenes from the Oregon population are most similar to var. *menziesii* from Olympic NP, WA. Additional research is needed to resolve this problem.

ACKNOWLEDGEMENTS

Thanks to Tonya Yanke for lab assistance and to Martha González and Abraham Torres for field assistance. This research was supported in part with funds from Baylor University.

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A new species of *Heliomeris* (Asteraceae: Heliantheae) from Oaxaca, Mexico

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ABSTRACT

A novel taxon, ***Heliomeris serboana* B.L. Turner sp. nov.**, is described from pine forests of Oaxaca, Mexico. It is closely related to the more northern *H. multiflora* Nutt., but differs by numerous characters, including habit, foliage and structure of the heads. A photograph of the type is provided, along with a map showing its distribution and that of *H. multiflora*.

Published on-line: www.phytologia.org *Phytologia* 95(1): 103- (Feb. 1, 2013).

KEY WORDS: Asteraceae, Heliantheae, *Heliomeris*, Mexico, Oaxaca

Routine identification of a large assemblage of Comps from Oaxaca, Mexico, gathered by SERBO has occasioned the present paper. The novelty was an unexpected find since I recently provided a treatment of *Heliomeris* for Mexico (Turner 2012) and the taxon was not accounted for among the large assemblage of plants studied; it is a pleasure to name the novelty for the organization that founded their assemblage, SERBO.

HELIOMERIS SERBOANA B.L. TURNER, sp. nov. Fig. 1

Diffusely branched herbs, or shrublets, to 1 m high. **Mid-stems** glabrous or nearly so. **Leaves** opposite throughout, 10-15 cm long, 3-5 cm wide; petioles slender, 0.5-2.0 cm long; blades ovate, 3-nervate from the very base, glabrous above and below, or nearly so; margins weakly serrate, if at all, the apices acuminate. **Capitulescences** both terminal and axillary, arranged in 5-15 headed cymose panicles, the ultimate peduncles 4-8 mm long. **Heads** 6-8 mm high, 3-5 mm wide (rays excluded). **Involucral bracts** (outer): 4-5, ca 3 mm long; inner bracts, 6-7 mm long. Receptacle convex, ca 1.5 mm across, paleate. **Ray florets** 3-5, neuter; corollas yellow, the rays 5-8 mm long. **Disk florets**, ca 10 to a head; corollas yellow, ca 4 mm long, sparsely pubescent; throat ca 1 mm long; lobes 5, ca 0.5 mm long. **Achenes** ca 3 mm long, epappose, glabrous, striate.

TYPE: MEXICO. OAXACA: Distrito, Sola de Vega; Mpio. Santiago Textitlan, “La Yerba Santa.” Pine forests, ca 2456 m, 16 39 43.5 N, 97 15 53.4 W, 19 Feb 2007, *Maria Ester Jacob Salinas 1602* (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: [essentially same locality as TYPE]: “A u lado de la Hierba Santa.” ca 1708 m, 19 39 53.5N, 97 16 10.2 W, 19 Feb 2007, *Salinas 1632* (TEX). “El Arroyo de la Cruz. Bosque de pino. Orilla de arroyo o rio.” ca 2692 m, 16 41 25.6 N, 97 15 54.8 W, 24 Feb 2007, *Salinas 1658* (TEX).

The type is described as an “arbusto,” 1 m high; the additional collection is described as a “Bejuco [a vine].” Both collections appear to be suffruticose herbs or shrublets

The following key to the Mexican species of *Heliomeris*, as modified from Turner (2012), should serve to identify members of the complex as currently known:

1. Leaves and stems coarsely hispid-pubescent throughout, the longer hairs 1.5-2.0 mm long; a relatively rare species of wet places or standing water; Son, Chi.....**H. hispida**
1. Leaves and stems not as above, at least some, or most of the hairs softer and shorter, 0.5-1.5 mm long**(2)**
2. Leaf blades broadly ovate to deltoid, 1-2 times as long as wide, the margins serrate; se Pue and adjacent Oax**H. obscura**
2. Leaf blades lanceolate to linear-lanceolate, 3-8 times as long as wide, the margins entire or nearly so; widespread**(3)**
3. Leaves 2-5 cm wide; ray florets 3-5; disc florets ca 10 per head; Oax.....**H. serboana**
3. Leaves 0.5-1.5 cm wide; ray florets 8-15; disc florets numerous per head; not known in Oax.**H. multiflora**

ACKNOWLEDGEMENTS

My research colleague, Jana Kos, provided helpful editorial input, for which I am grateful. The distribution map (Fig. 2) is based upon specimens on file at LL-TEX, except for the collections of *H. multiflora* from the vicinity of San Cristobal de Las Casas, Chiapas, which are based upon specimens referred to as *H. longifolia* by Strother (1999).

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Fig. 1. *Heliomeris serboana* (Isotype: TEX).

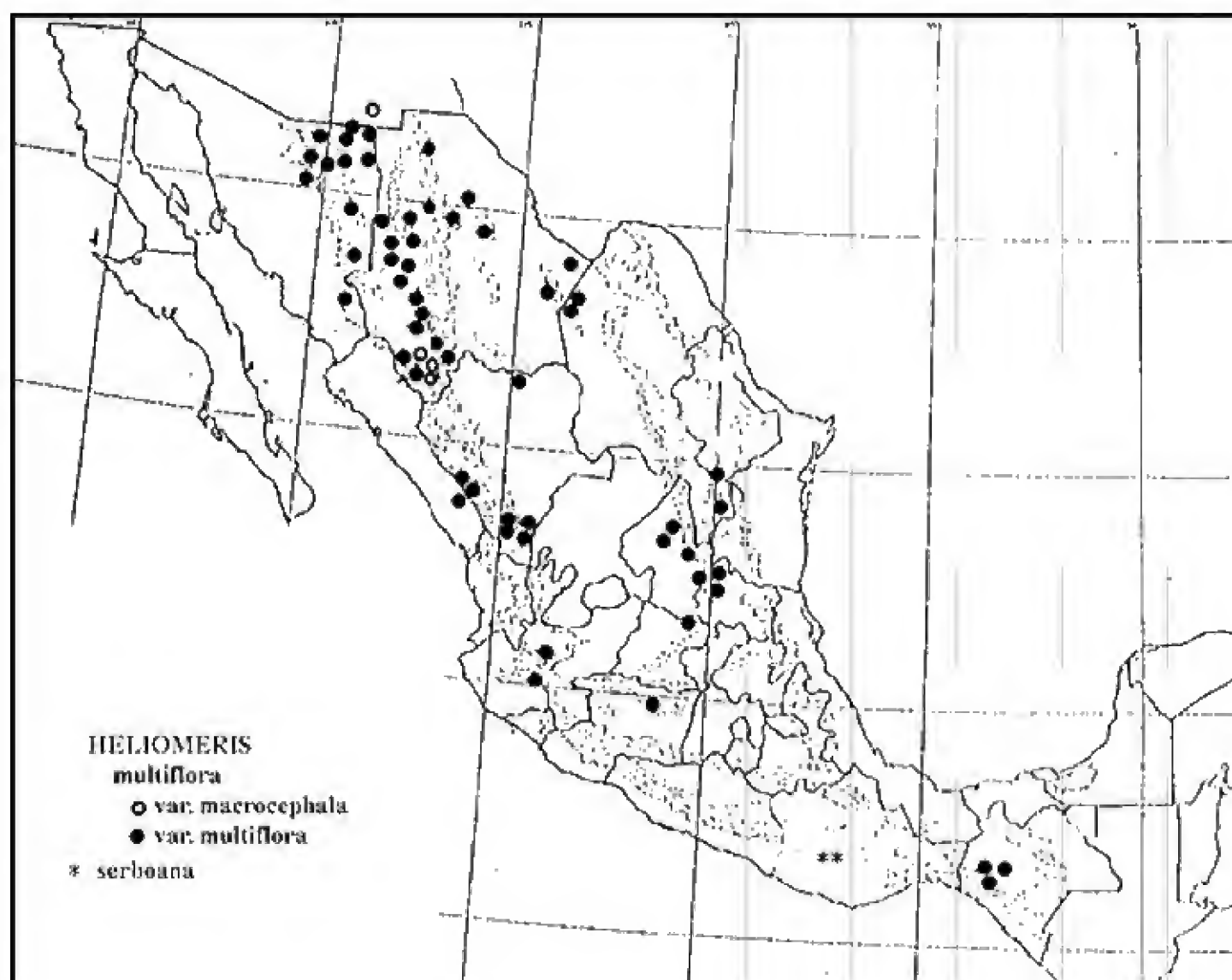


Fig. 2. Distribution of *Heliomeris multiflora* and *H. serboana* in Mexico.

Hybridization between *Juniperus grandis*, *J. occidentalis* and *J. osteosperma* in northwest Nevada II: Terpenes, Buffalo Hills, Northwestern Nevada

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ABSTRACT

The volatile leaf oils of *J. grandis*, *J. occidentalis*, *J. osteosperma* and putative hybrids in northwestern Nevada along hwy 446 (Buffalo Hills) were analyzed. No evidence of hybridization involving *J. grandis* was found among the presumed hybrids and backcrosses of *J. occidentalis* and *J. osteosperma*. In fact, the terpene data was relatively uniform, suggesting that the complex concerned is a stabilized hybrid population. No plants having typical terpenes, of *J. occidentalis* and *J. osteosperma* were found. The terpene analysis seems in agreement with the haplotype data of Terry (2010).

Published on-line: www.phytologia.org *Phytologia* 95(1): 107-114 (Feb. 1, 2013).

KEY WORDS: *J. grandis*, *J. occidentalis*, *J. osteosperma*, hybridization, Cupressaceae, terpenes, Buffalo Hills, northwestern Nevada.

Vasek (1966) published the seminal paper on hybridization among species of *Juniperus* in north-western Nevada. Based on exhaustive field work and morphological data, he concluded that *J. occidentalis* and *J. osteosperma* were hybridizing across a large area of northwestern Nevada. His careful observations and analyses were confirmed by Terry et al. (2000) and Terry (2010), who found cpDNA (trnL-trnF, trnS-trnG) haplotypes of *J. occidentalis* in Nevada populations of *J. osteosperma*, with lower frequencies occurring in Utah, Colorado, and Wyoming. Subsequently, Terry (2010) analyzed trnL-trnF and trnS-trnG (cpDNA) haplotypes and reported similar results (Fig. 1). He found that all samples of *J. occidentalis* from Oregon were uniform in having only haplotype 9 (Fig. 1). However, the NV 446 population had two of the *J. occidentalis* haplotypes and yet two other haplotypes (Fig. 1).

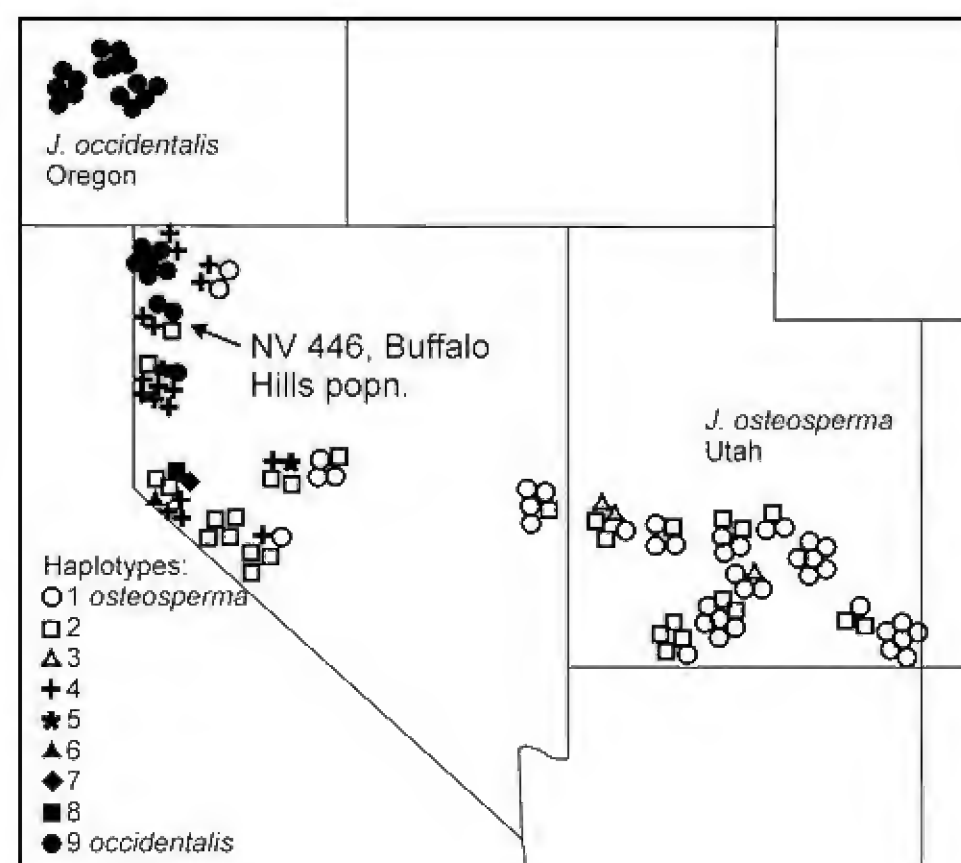


Figure 1. Distribution of haplotypes (trnL-trnF and trnS-trnG) in *J. occidentalis* and *J. osteosperma* (based on Terry, 2010).

J. grandis, *J. occidentalis*, *J. osteosperma* occupy generally allopatric ranges (Fig. 2), with *J. grandis* favoring granitic outcrops in the high Sierra, *J. occidentalis* growing on lava beds at lower elevations in northern California and Oregon, and *J. osteosperma*, preferring the intermediate elevations in the Basin and Range region of Nevada, Utah and adjacent states; a fourth species, *J. californica*, grows in the Mojave desert foothills of southern California, thence northward in the central valley foothills (Adams 2011). Adams (2012a, b) found that *Juniperus grandis* and *J. occidentalis* appear to hybridize in the Beckwourth, CA area (Fig. 2) but, otherwise, no evidence of gene flow between these species was found (Adams and Kaufmann, 2010).

Juniperus grandis and *J. osteosperma* have been shown (Adams 2013) to hybridize at the Leviathan Mine area of western Nevada (Fig. 2). It is interesting to note that plants typical of both parents were found, along with quite intermediate individuals, and those appearing to be backcrossed to *J. osteosperma*, but not to *J. grandis* (Fig. 2).

The nw NV hwy 446 population, sampled by Terry (2010, popn. 18, Buffalo Hills) is in an area of sympatry between *J. occidentalis* and *J. osteosperma* (Fig. 3) and subject to ancestral as well as possible current hybridization. Analysis of the terpenes from plants of the NV hwy 446 (Buffalo Hills) population is the purpose of this paper.

MATERIALS AND METHODS

Plant material: *J. grandis*, Adams 11963-11967, Jct. US 50 & CA 89, 38° 51.086' N, 120° 01.244' W, 1937 m, Meyers, El Dorado Co.; CA; Adams 11968-11972, 16 km w of Sonora Jct., on CA. 108, 38° 18.289' N, 111° 35.598' W, 2585 m, Tuolumne Co.; CA; *J. osteosperma*, Adams 1689-1699, 1701-1705, on US 6, Thistle, 40° 00' 6.9" N, 111° 29' 4.6" W, 1650 m, Utah Co., UT; Adams 12067-12071, 4 km n of Sedona, AZ, at Grasshopper Point, on Alt US 89, 34.888° N, 111.733° W, 1380m, Coconino Co., AZ; Adams 10272-10276, on NV157, Charleston Mtns., 36° 16.246' N, 115° 32.604' W, 1795 m, Clark Co., NV; Adams 11122-11124, Hancock Summit, mile 38 *occidentalis* (in part) and *J. osteosperma* on US 375, 37° 26.404' N, 115° 22.703' W, 1675 m, (in part) with Leviathan mine population noted. Lincoln Co. NV; Adams 11125-11127, McKinney Tanks Summit on US 6, 38° 07.005' N, 116° 54.103' W, 1933 m, Nye Co., NV; Adams 11134-36, 8 km s of Bridgeport, on US395, 38° 12.639' N, 119° 13.846' W, 2004 m, Mono Co., CA; Adams 11141-11143, 13 km w of Elko, on I 80, 40° 45.598' N, 115° 55.942' W, 1535 m, Elko Co., NV; Adams 11144-11146, 8 km e of Wells, on I 80, 41° 06.533' N, 114° 51.441' W, 1876 m, Elko Co., NV; Adams 11960-11962, 56 km

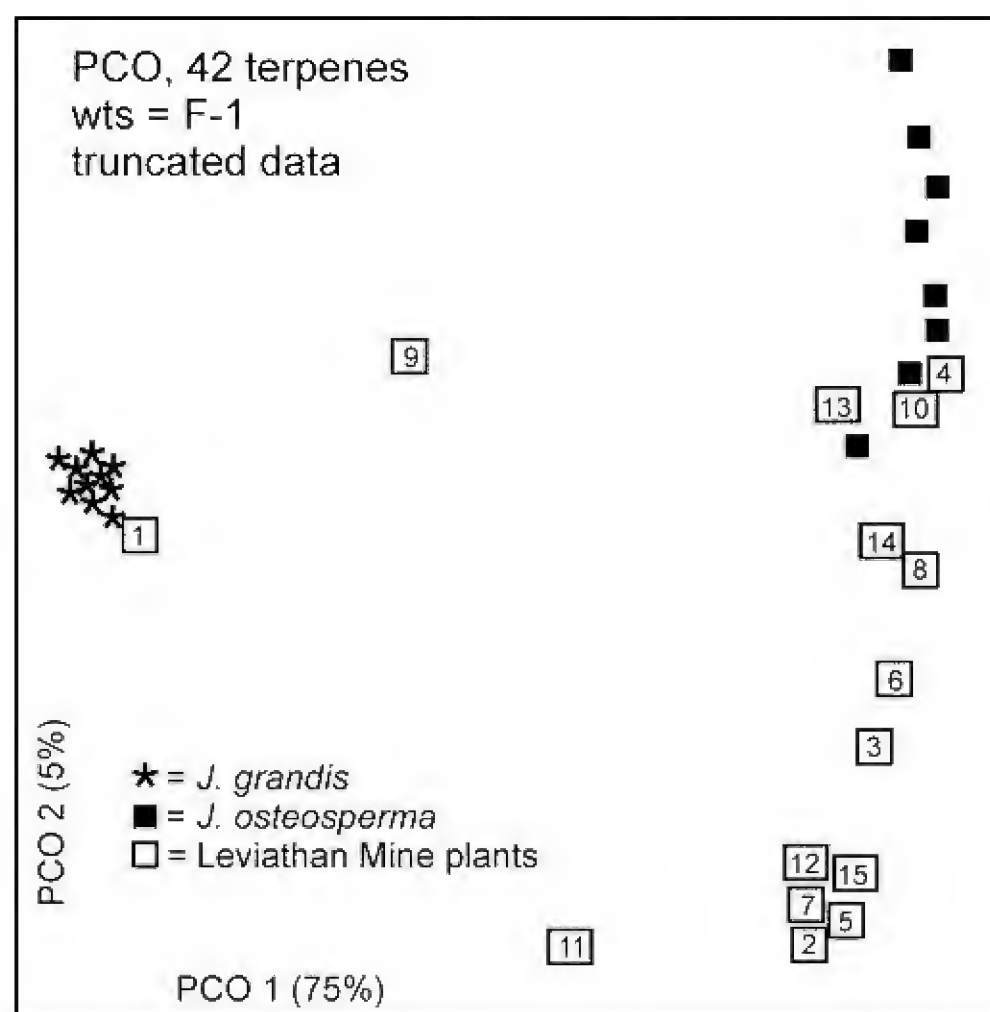


Figure 2. PCO of *Juniperus* from the Leviathan Mine with plants of the parental species (from Adams 2013).

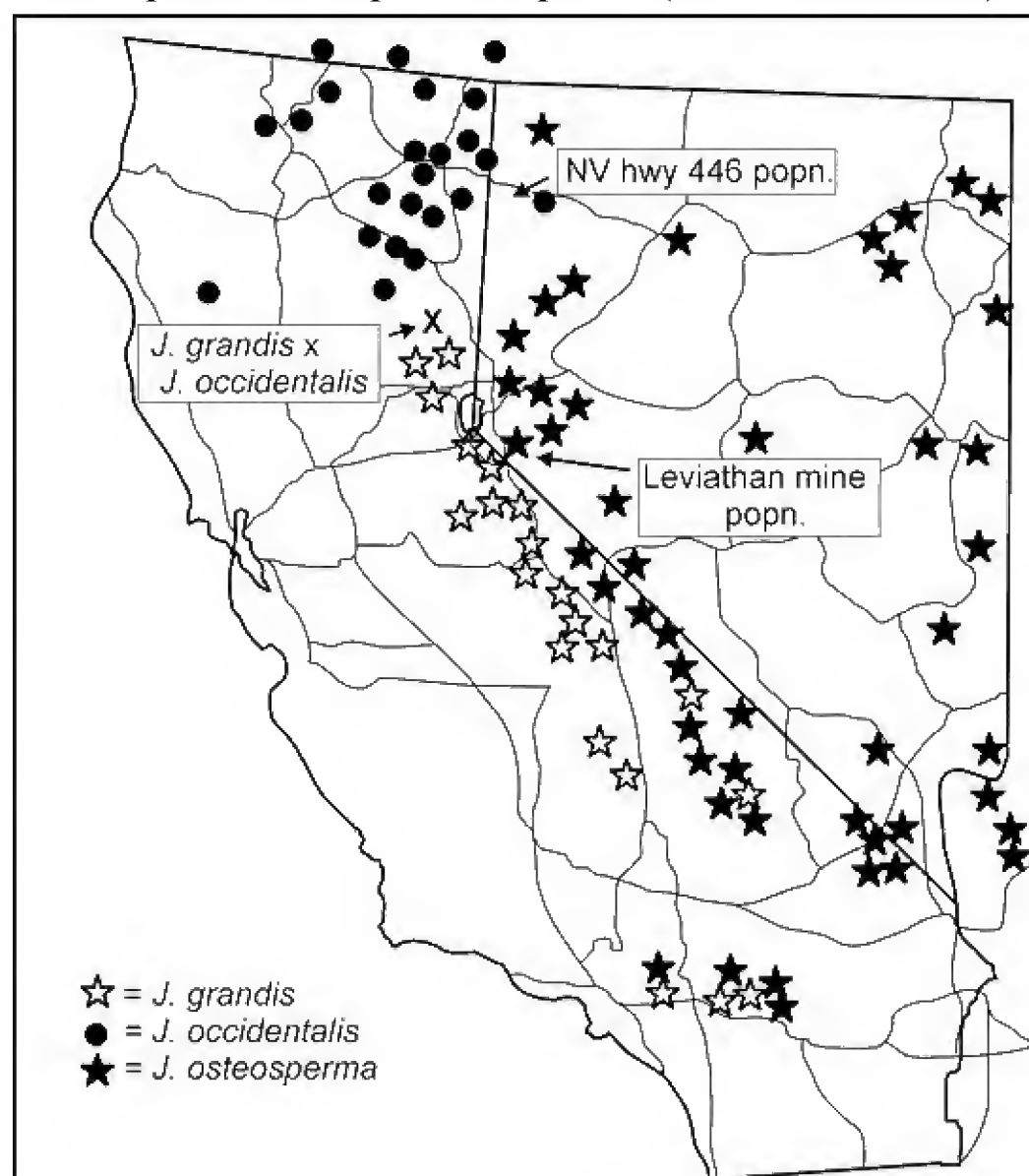


Figure 3. Distribution of three western junipers and the location of the NV hwy 446 population of this study. The eastern distribution of *J. osteosperma* is not shown.

n of Reno, NV; on US 395, 39° 54.458' N, 120° 00.322' W, 1383 m, Lassen Co., CA; *Adams 11973-11977*, 10 km n of CA 168 on White Mtn. Rd., 37° 20.143' N, 118° 11.346' W, 2607 m, Inyo Co., CA; *Adams 11978-11982*, Mahogany Flats Campground, Panamint Mtns., 36° 13.783' N, 117° 04.102' W, 2477 m, Inyo Co., CA, *Adams 12323-12327*, Basin, San Bernardino Mtns., 34° 16.910' N, 116° 45.306' W, 1820 m, San Bernardino Co., CA, *Adams 12210-12214*, ca. 1 km e of CA 18, ca. 16 km s of jct CA 18 & CA 247, n slope San Bernardino Mtns., 34° 21.213' N, 116° 50.607' W, 1393 m, San Bernardino Co., CA, *Adams 12215-12219*, on I15, at Bailey Rd., 35° 27.938' N, 115° 31.709' W, 1431 m, San Bernardino Co., CA. ***J. occidentalis***, *Adams 11940-11942*, 12 km e of Jct. WA 14 & US 97 on WA 14, 45° 44.392' N, 120° 41.207' W, 170 m, Klickitat Co.; WA, *Adams 11943-11945*, 2 km s of jct. US 97 & US 197 on US 97, 38 km ne of Madras, OR; 44° 53.676' N, 120° 56.131' W, 951 m, Wasco Co., OR; *Adams 11946-11948*, 3 km sw of Bend, OR; on OR 372, 44° 02.390' N, 121° 20.054' W, 1132 m, Deschutes Co., OR; *Adams 11949-11951*, 32 km e of Bend, OR on OR 20, shrubs, 0.5 - 1m tall, 43° 53.922' N, 120° 59.187' W, 1274 m, Deschutes Co., OR; *Adams 11952-11954*, 14 km e of Jct. OR66 & I 5, on OR66, 42° 08.044' N, 122° 34.130' W, 701 m, Jackson Co., OR; *Adams 11957-11959*, on CA 299, 10 km e of McArthur, CA, 41° 05.313' N, 121° 18.921' W, 1091 m, Lassen Co., CA; *Adams 11995-11998* (*Kauffmann A1-A3, B1*), Yolla Bolly-Middle Eel Wilderness, 40° 06' 34" N, 122° 57' 59" W, 1815- 2000 m, Trinity Co., CA, *Adams 12342-12346*, 19 km WSE of Susanville, CA, on CA 36, 40° 22.178' N, 120° 50.211' W, 1570 m, Lassen Co., CA, *Adams 12347-12351*, on US 395, 5 km n of Madeline, 41° 05.867' N, 120° 28.456' W, 1695 m, Lassen Co., CA. **northwestern Nevada, hwy 446 population:** *Adams 12352-12366*, at mile 98, NV hwy 446 [=Terry (2010) popn.#18, Buffalo Hills], 40° 53.104' N; 119° 36.212' W, 5667 ft. Voucher specimens are deposited in the herbarium, Baylor University (BAYLU).

Isolation of Oils - Fresh leaves (200 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

Chemical Analyses - Oils from 10-15 trees of each taxon were analyzed and average values reported. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967). Principal components analysis (PCA) follows the formulation of Veldman (1967).

RESULTS AND DISCUSSION

To determine if *J. grandis* might be involved in hybridization in this population, ANOVA was performed on the leaf oil components of *J. grandis*, *J. occidentalis* and *J. osteosperma* (data from Adams 2013). The resulting F ratios (from ANOVA) were used as character weights (i.e., F-1.0) to produce a matrix of similarities that was factored by PCO and ordinated (Fig. 4). From this ordination, there does not appear to be any of the putative hybrids that are tending to cluster with *J. grandis*, lending credence that *J. grandis* is not genetically involved in the nw NV, hwy 446 population. Of course, ancient hybridization and introgression might have had a very subtle effect on the leaf oils, and such events can not be eliminated.

The leaf oil compositions of *J. occidentalis* and *J. osteosperma* have 19 highly significant, and 2 significant differences (Table 1). Interestingly, two of the largest components, sabinene (11.5 - 12.9%)

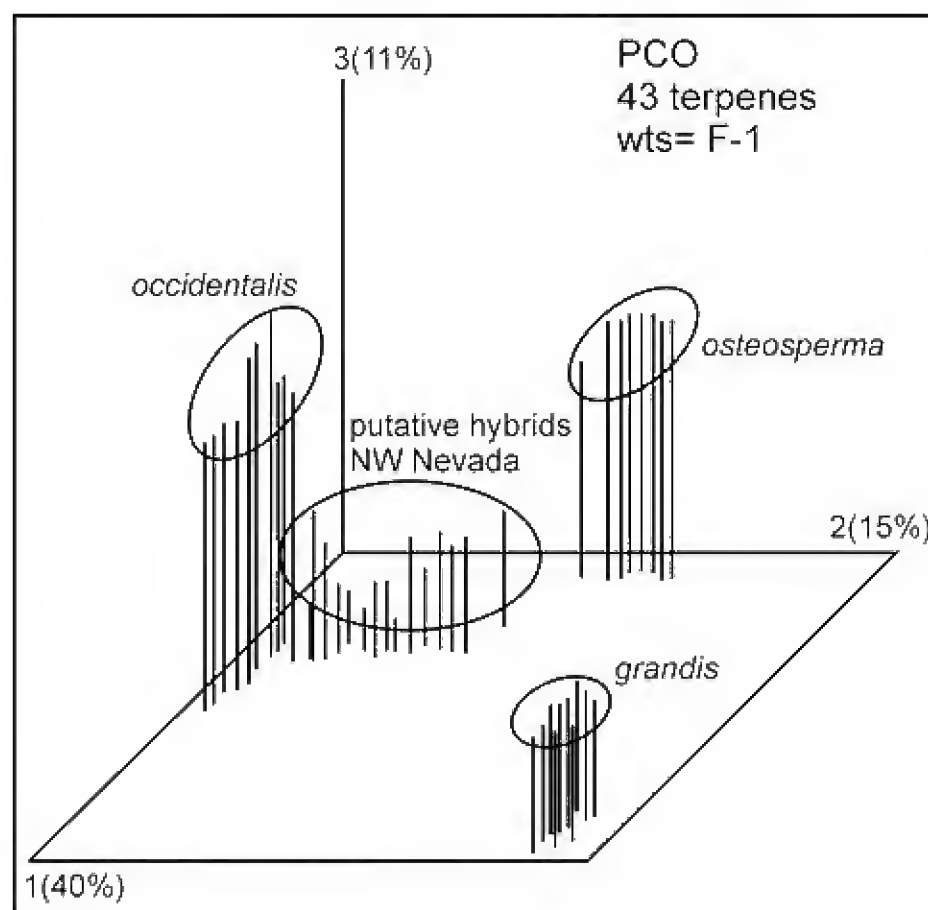


Figure 4. PCO of *J. grandis*, *J. occidentalis*, *J. osteosperma* and putative hybrids from NW NV.

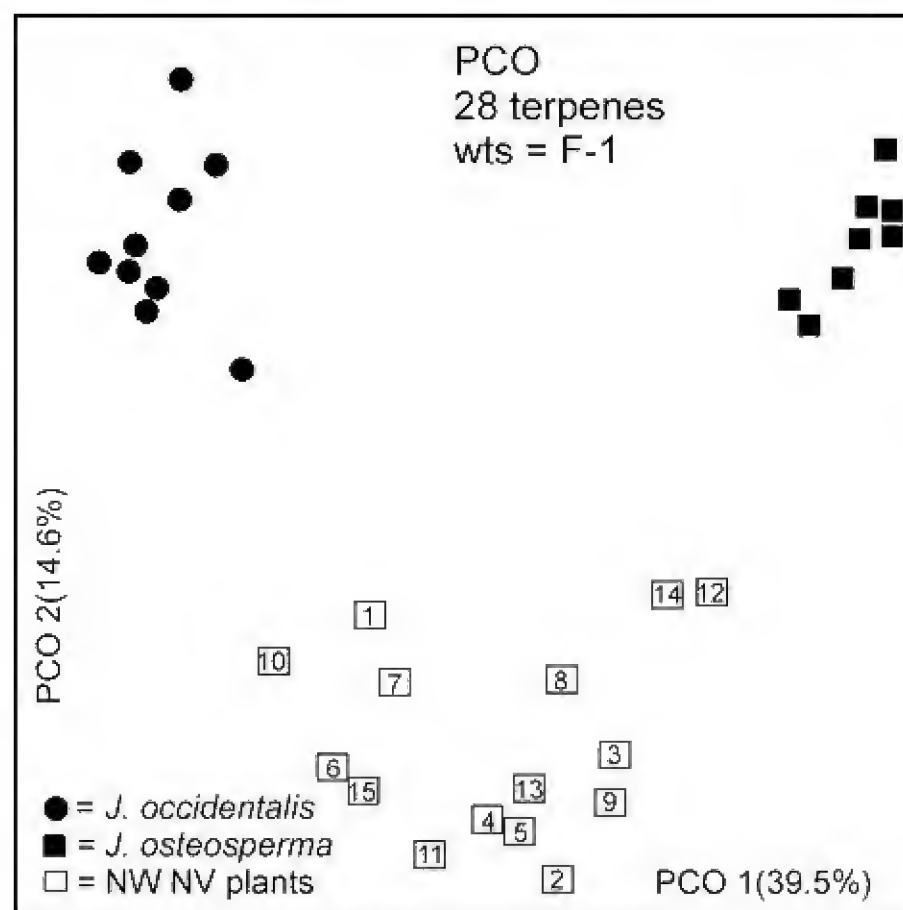


Figure 5. PCO of *J. occidentalis*, *J. osteosperma* and plant from NW NV. see text for discussion.

and bornyl acetate (13.3 - 13.6%) were not significantly different. Sabinene is notable in being found in higher concentration in the putative hybrids than in the parents (14.9 - 30.5%). Twenty nine of the compounds were used to compute similarity measures using character weights of F-1.0 (F from ANOVA between the parents). Factoring the similarity matrix by PCO gave eigenroots that accounted for 39.5, 14.6, 5.8, 5.4 and 4.46% of the variance among plants. Ordinating the data on the first two axes shows *J. occidentalis*, *J. occidentalis*, and the plants from nw NV as a third group (Fig. 5). Within the NV plant group, individuals 12 and 14 (Fig. 5) appear to have oils intermediate to *J. osteosperma*; whereas plants 1, 7, and 10 show an affinity to *J. occidentalis*. It may be that these plants represent backcrossed individuals, or some linkage group as found in *Cryptomeria japonica* artificial hybrids (Adams and Tsumura, 2012), or this may be a case of dominant/ recessive terpenes influencing the similarities as reported in Douglas fir (Adams and Stoehr, 2013). However, in the case of Douglas fir synthetic hybrids, the dominant terpenes place a number of F₁ hybrids near one parent, not both parents as in the present case. So it seems more likely that plants 1, 10, 12, and 14 are backcrossed individuals.

It is interesting to compare the patterns in the Leviathan mine (Fig. 2) and the nw NV population (Fig. 5). The Leviathan mine area clearly contained both parents as their oils were typical of *J. grandis* and *J. osteosperma*. In contrast, in the nw NV population, no plants clustered with *J. occidentalis* or *J. osteosperma*. Of course, as the purpose of this study was to document hybridization, it is likely that the author collected from those plants showing intermediate morphology. Table 2 shows field notes on the 15 trees sampled and the subsequent terpene assessment of their identities. Several of the plants field identified as *J. osteosperma* (2, 4, 7) had hybrid terpene patterns (Fig. 5). Plant 8, field-identified as *J. occidentalis*, was found to be quite intermediate in its terpenes (Fig. 5) and another plant identified as *J. occidentalis* ? (#10), appears to be a backcross to *J. occidentalis*; in short, these two species are difficult to identify in nw NV, and it is likely that specimens have been misidentified in the past.

Table 1. Morphological observations on plants of the NV 446 (Buffalo Hills) population.
bc occ = backcross to *J. occidentalis*, bc ost = backcross to *J. osteosperma*.

occid x osteo, Washoe Co, NV

coll#(id #)	morph, in field	terpenes, this study	monecious?	# stems	glands visible	ruptured glands
occid	occid	occid	50% monec	1(-3)	visible	ruptured
osteo	osteo	osteo	90% monec	several	not conspicuous	not-ruptured
12352 (1)	bc occ	bc occ	monec.	1 stems	not visible	few ruptured
12353 (2)	osteo	hybrid	female	8 stems	not visible	v. few ruptured
12354 (3)	hybrid	hybrid	monec	5 stems	visible	not ruptured
12355 (4)	osteo	hybrid	female	3 stems	not visible	not ruptured
12356 (5)	occid	hybrid	female	1 stems	visible	ruptured
12357 (6)	hybrid	bc occ	female	10 stems	visible	ruptured
12358 (7)	osteo	bc occ	male	10 stems	not visible	not ruptured
12359 (8)	occid	hybrid	monec	1 stems	visible	ruptured
12360 (9)	hybrid	hybrid	monec	3 stems	visible	ruptured
12361 (10)	occid?	bc occ	female	1 stems	not visible	not ruptured
12362 (11)	hybrid	hybrid	monec	5 stems	visible	ruptured
12363 (12)	hybrid	bc ost	monec	4 stems	visible	ruptured
12364 (13)	hybrid	hybrid	female	1 stems	visible	ruptured, white exud
12365 (14)	hybrid	bc ost	female	5 stems	visible	ruptured, white exud
12366 (15)	hybrid	bc occ	monec	6 stems	visible	ruptured, white exud

CONCLUSIONS

The nw NV population along hwy 446 (Buffalo Hills of Terry, 2010) appears to be composed of chiefly hybrids and possible backcrosses, in contrast to the Leviathan Mine population that contains both parents, hybrids and backcrosses to only *J. osteosperma*. The uniformity of the nw NV population seems unusual for a hybrid swarm, and suggests that it may be a stabilized hybrid population. Additional research will be needed to elucidate this unusual pattern.

ACKNOWLEDGEMENTS

Thanks to Billie Turner for proofing the manuscript. This research was supported with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

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Table 2. Leaf essential oil compositions for *J. occidentalis* and *J. osteosperma* plus #2, 5 (putative hybrids), #10 (putative backcross to *J. occidentalis*) and #12 (putative backcross to *J. osteosperma*, (see Fig. 4). Compounds in boldface were used in PCO analysis. F from ANOVA between *J. occidentalis* and *J. osteosperma*. significance: * = 0.05; ** = 0.01; ns = not significant, nt = not tested in ANOVA.

KI	compound	occid	osteo	#2	#5	#10	#12	F ex ANOVA
921	tricyclene	0.8	0.5	0.4	0.4	0.3	0.6	2.5 ns
924	α-thujene	1.2	0.6	0.6	0.7	0.9	0.5	23.1 **
932	α-pinene	3.6	4.2	2.9	2.4	2.7	3.6	1.2 ns
945	α -fenchene	t	-	-	t	t	0.2	nt
946	camphene	0.8	0.7	0.4	0.5	0.4	0.7	1.2 ns
953	thuja-2,4-diene	t	t	-	-	-	-	nt
961	verbenene	0.9	t	-	t	-	-	nt
969	sabinene	12.9	11.5	22.1	23.0	30.5	14.9	ns
974	β -pinene	0.4	0.2	0.5	0.1	0.1	0.4	ns
988	myrcene	1.9	1.4	2.5	2.0	2.6	2.6	2.8 ns
1001	δ -2-carene	t	-	-	-	-	-	nt
1002	α-phellandrene	0.8	0.3	0.2	0.1	0.2	0.1	23.3 **
1008	δ-3-carene	1.6	-	-	0.3	-	5.4	7.4 **
1014	α-terpinene	1.9	1.6	2.5	1.8	2.8	1.4	2.2 ns
1020	p-cymene	10.4	2.4	0.5	0.6	0.6	0.4	23.5 **
1024	limonene/β-phellandrene	1.2	2.2	5.1	3.7	3.5	5.6	23.4 **
1044	(E)- β -ocimene	0.1	t	0.7	0.4	0.4	0.8	ns
1054	γ-terpinene	3.2	2.5	4.2	3.0	4.5	2.3	3.2 ns
1065	cis-sabinene hydrate	0.8	1.1	1.9	1.7	1.4	1.2	5.2 ns
1086	terpinolene	1.5	1.3	1.7	1.2	1.1	1.9	2.9 ns
1095	linalool	0.5	-	t	t	t	0.1	nt
1098	trans-sabinene hydrate	0.6	1.4	1.5	1.1	1.1	1.0	227.5 **
1118	cis-p-menth-2-en-1-ol	0.7	0.6	0.9	0.6	0.8	0.6	ns
1136	trans-p-menth-2-en-1-ol	0.8	-	0.5	0.2	0.5	-	227.6 **
1141	camphor	1.4	21.3	4.9	6.9	0.8	21.7	108.6 **
1145	camphene hydrate	0.2	1.4	1.2	1.0	0.4	1.2	336.5 **
1154	sabina ketone	0.4	1.1	0.1	0.2	0.1	0.3	68.4 **
1165	borneol	1.1	4.8	0.5	0.5	0.1	1.5	22.3 **
1166	coahuilensol	1.2	t	t	3.3	0.2	0.4	35.6 **
1174	terpinen-4-ol	7.6	9.8	12.0	8.4	12.0	6.7	5.2 *
1179	p-cymen-8-ol	0.5	0.5	t	-	t	0.2	nt
1186	α -terpineol	0.4	0.4	0.7	0.5	0.6	0.5	ns
1195	myrtenol	-	0.2	t	t	t	t	nt
1195	cis-piperitol	0.2	0.3	0.3	0.3	0.2	0.1	nt
1204	verbenone	-	0.2	-	-	-	-	nt
1207	trans-piperitol	0.3	0.3	0.5	0.3	0.3	0.4	nt
1215	trans-carveol	-	0.6	-	-	-	-	nt
1219	coahuilensol, me-ether	1.1	0.2	t	6.8	0.5	0.4	ns
1230	trans-chrysanthenyl ac.	-	-	0.8	-	-	-	nt
1238	cumin aldehyde	0.2	0.3	-	-	-	-	nt
1239	carvone	-	0.6	-	-	-	-	nt
1249	piperitone	0.2	t	t	-	-	-	nt
1257	methyl citronellate	-	-	0.3	-	t	-	nt
1283	α -terpinen-7-al	-	0.2	-	-	-	-	nt
1284	bornyl acetate	13.6	13.3	14.8	15.5	10.3	13.7	0.01 ns
1298	carvacrol	0.4	t	0.2	0.3	0.2	0.4	nt
1318	149,69,91,164, phenolic	-	0.4	0.8	0.4	2.9	2.6	33.8 **
1318	methyl geranate	1.6	-	-	-	-	-	nt
1325	p-mentha-1,4-dien-7-ol	t	0.5	-	-	-	-	nt
1387	β -bourbonene	0.2	-	-	-	-	-	nt

KI	compound	occid	osteo	#2	#5	#10	#12	F ex ANOVA
1429	cis-thujopsene	0.9	0.7	-	-	-	-	nt
1451	trans-muuro-la-3,5-diene	0.1	-	-	-	-	-	nt
1465	cis-muuro-la-4,5-diene	0.1	-	-	-	-	-	nt
1468	pinchotene acetate	1.0	0.1	t	2.2	-	-	7.2 *
1475	trans-cadina-1(6),4-diene	0.3	-	-	-	-	-	nt
1478	γ -muurolene	0.8	-	-	-	-	-	nt
1484	germacrene D	0.3	-	-	-	-	-	nt
1493	trans-murrola-4(14),5-diene	0.4	-	-	-	-	-	nt
1493	epi-cubebol	0.4	-	-	-	-	-	nt
1500	α -muurolene	1.1	t	t	t	t	-	nt
1513	γ-cadinene	1.9	t	0.5	0.3	0.7	0.1	34.3 **
1518	epi-cubebol	0.4	-	-	-	-	-	nt
1522	δ-cadinene	2.4	0.5	0.8	0.8	0.7	0.4	15.2 **
1537	α -cadinene	0.4	-	-	-	-	-	nt
1544	α -calacorene	0.3	-	-	-	-	-	nt
1548	elemol	0.2	1.3	0.7	0.3	6.2	0.6	52.3 **
1574	germacrene-D-4-ol	0.5	0.1	1.0	0.7	0.4	0.4	21.3 **
1582	caryophyllene oxide	-	t	-	-	-	-	nt
1586	gleenol	0.3	-	-	-	-	-	nt
1607	β -oplophenone	0.3	t	0.7	0.3	0.2	0.2	nt
1608	humulene epoxide II	-	t	-	-	-	-	nt
1618	1,10-di-epi-cubenol	0.2	-	-	-	-	-	nt
1627	1-epi-cubenol	1.6	-	t	0.4	0.5	-	nt
1630	γ -eudesmol	-	0.2	-	-	0.6	-	nt
1638	epi-α-cadinol/muurolol	0.7	t	1.5	0.8	1.2	0.6	55.1 **
1644	α -muurolol	0.2	-	0.1	0.1	0.2	t	nt
1649	β -eudesmol	-	0.2	0.1	t	0.7	t	nt
1652	α -eudesmol	-	0.2	t	-	1.2	-	nt
1652	α-cadinol	1.3	0.3	2.4	1.1	1.3	1.0	43.7 **
1739	oplopanone	-	t	0.2	t	t	t	nt
1870	198, 205, 220, 149	-	-	0.3	0.5	0.4	0.4	nt
1987	manoyl oxide	2.2	-	t	t	t	0.1	nt
2009	epi-13-manoyl oxide	t	-	-	-	-	-	nt
2312	abieta-7,13-dien-3-one	-	0.1	-	-	-	t	nt

KI = linear Kovats Index on DB-5 column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Eucnide lobata* (Loasaceae), first record for the U. S. A.*Billie L. Turner**

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ABSTRACT

Eucnide lobata, previously known only from northeastern Mexico, has now been collected in southern Texas, along the Rio Grande in Starr County (just SE of Rio Grande City). A voucher specimen of the collection is cited and pictures of the living plants in the field are provided. A map showing its distribution in Mexico and Texas is included. Published on-line: www.phytologia.org *Phytologia* 95(1): 115-117 (Feb. 1, 2013).

KEY WORDS: Loasaceae, *Eucnide*, *E. lobata*, Texas, Starr County, Mexico, U.S.A.

About five years ago, a specimen of the genus *Eucnide* was sent by the second author to the University of Texas Herbarium (TEX) for deposition in that collection. Having worked on the genus *Eucnide*, the first author recognized immediately that it was a new record for the state, recorded it so, and provided a new map for the taxon in his soon to be updated *Atlas of the Vascular Plants of Texas* (Turner et al. 2003). There it remained until the Curator of the Herbarium suggested that it be called to the attention of plant enthusiasts of the state, hence the present paper. The specimen concerned follows:

TEXAS. STARR CO.: "Hill with large cross, just SE of Rio Grande City." Growing on limestone rocks, 25 Nov 2005, *Alfred Richardson & Ken King* 3350 (TEX).

The seminal taxonomic treatment of *Eucnide* has been that of Thompson and Ernst (1967). In this they distinguish *E. lobata* from *E. bartonioides* by the following couplet:

Corolla rotate, to about 12 mm long or 2 cm wide.....	E. lobata
Corolla open-funnelform and larger.....	E. bartonioides

Yet other characters serve to distinguish between the two taxa, but need not be enumerated here, since their distributions show clearly to what population systems each belongs (Maps 1 and 2).

The two collectors, in their exceptional text, *Plants of Deep South Texas* (Richardson and King 2011), accounted for the species, provided an excellent colored photograph, but identified the taxon as *E. bartonioides*, which is perhaps its closest relative (Turner 2012). In their text the authors note that the plant grows "on limestone cliffs in protected places," a typical habitat for most of the species in *Eucnide*; nevertheless, actual outcrops of limestone do not occur along the Rio Grande in that immediate area and the substrate is probably the calichified rocky alluvium widespread there. The collectors also noted that the population concerned consisted of ca. 15 plants (cf. Figs 1, 2).

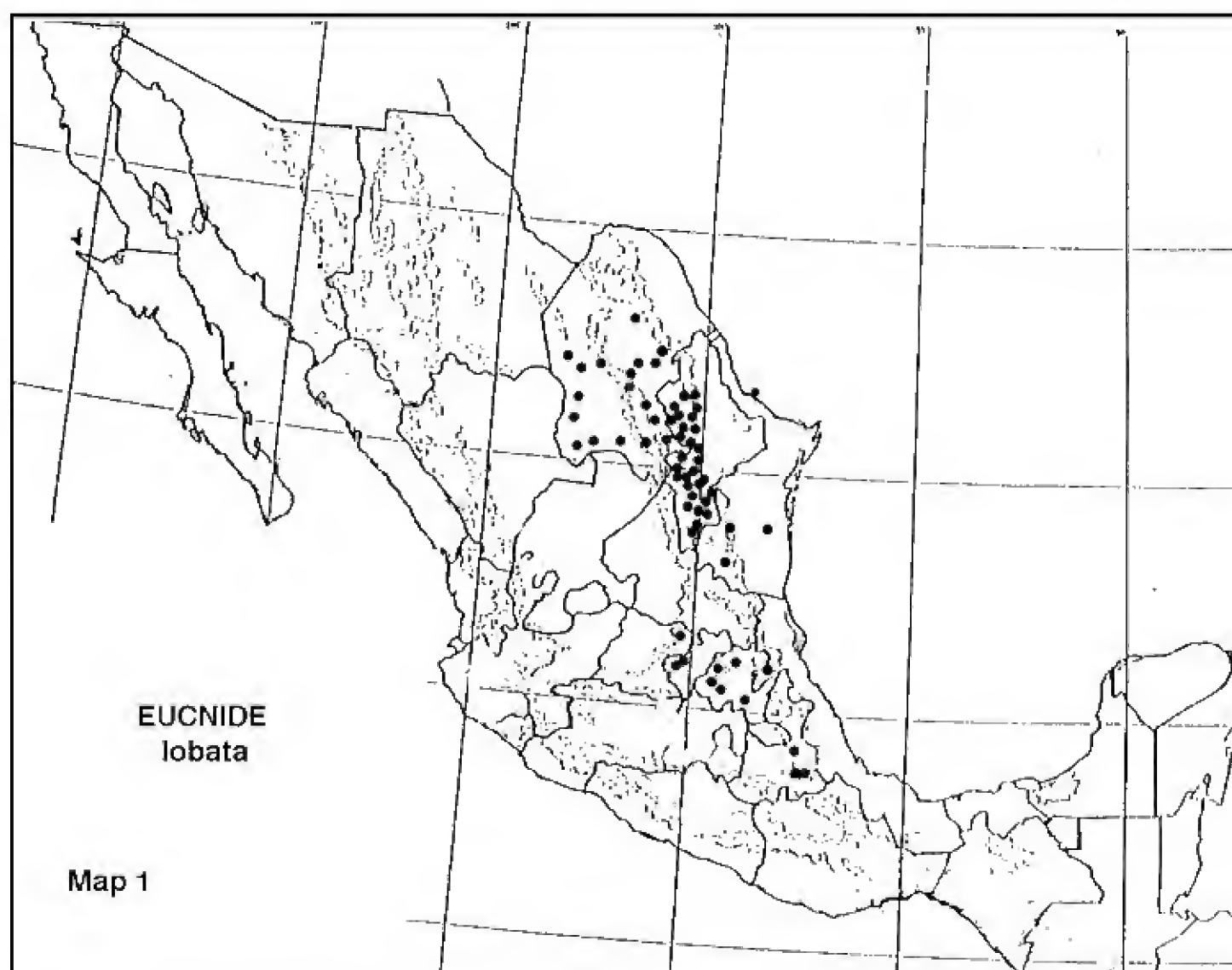
Examination of similar habitats in the region concerned might reveal yet other populations of the species concerned.

ACKNOWLEDGEMENTS

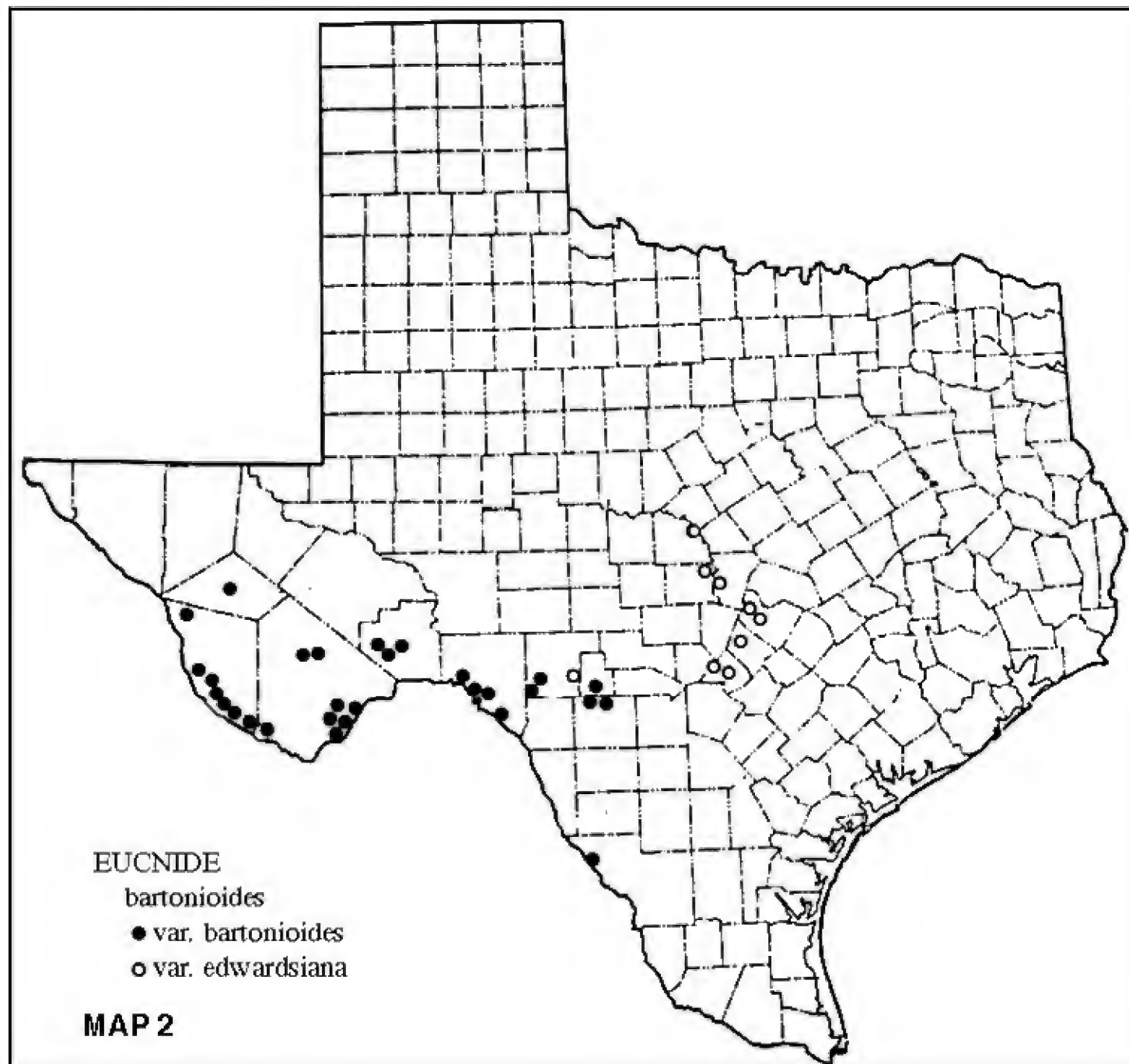
Thanks to the curator of TEX-LL, Tom Wendt, for helpful comments and suggesting that we compose the present paper.

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Map 1. Distribution of *Eucnide lobata*.



Map 2. Distribution of *E. bartonioides* in Texas.



Figs 1, 2. *Eucnide lobata* (field site).

The multi-seeded, entire leaf taxa of *Juniperus*, section *Sabina*: inclusion of *Juniperus microsperma***Robert P. Adams**Biology Department, Baylor University, Box 97388, Waco, TX
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ABSTRACT

A recent acquisition of a specimen of *Juniperus microsperma* from Tibet provided the opportunity to include that taxon in an analysis of the multi-seeded, entire leaf-margined taxa of *Juniperus* sect. *Sabina* based on nrDNA and four cpDNA regions. *Juniperus microsperma* was found to be very distinct, but most closely related to *J. erectopatens*. Published on-line: www.phytologia.org *Phytologia* 95(1): 118-121 (Feb. 1, 2013).

KEY WORDS: Taxonomy, *Juniperus*, section *Sabina*, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, *Juniperus microsperma*, *J. erectopatens*.

Adams and Schwarzbach (2012b) presented a robust analysis of the multi-seeded, entire leaf-margined taxa of *Juniperus* sect. *Sabina* based on nrDNA and four cpDNA regions, but no materials of *Juniperus microsperma* (W. C. Cheng & L. K. Fu) R. P. Adams from the type locality (Tibet) were available to the senior author for examination. However, recently, material of *Juniperus microsperma*, from near the type locality in Tibet, has become available for analysis of its leaf essential oil composition (Adams et al. 2013). The volatile leaf oil of *J. microsperma* from Song Zong, Xizang was very distinct and was dominated by sabinene (33.9%), pregeijerene B (16.3%), elemol (14.6%) and 8- α -acetoxyelemol (7.1%) with moderate amounts of terpinen-4-ol, germacrene D, and α - and β -eudesmols (Adams et al. 2013).

Previously, we have presented analyses of the serrate leaf taxa of *Juniperus*, sect. *Sabina* (Adams and Schwarzbach, 2011), all taxa of *Juniperus* sect. *Juniperus* (Adams and Schwarzbach, 2012a), the multi-seeded, smooth leaf *Juniperus* sect. *Sabina* (Adams and Schwarzbach, 2012b) and the turbinate *Juniperus* (Adams and Schwarzbach, 2012c). The purpose of the current study is to re-analyze all the multi-seeded, entire leaf-margined taxa of sect. *Sabina* using the most informative nuclear (nrDNA- ITS) and cpDNA regions (petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF) to include *J. microsperma* from Tibet. The pseudo-denticulate species, *J. phoenicea* (Adams, 2011) is included, as its placement is uncertain (Mao et al. 2010).

MATERIALS AND METHODS

Specimens used in this study (species, location, collection numbers): *J. microsperma*, Song Zong, Xizang (Tibet), China, *Jian-Quan Liu QTP-2011-201* (lab accession 13633; Voucher specimens for all collections are deposited at Baylor University Herbarium (BAYLU).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted by use of a Qiagen mini-plant kit (Qiagen,

Valencia, CA) as per manufacturer's instructions. *PCR amplification, sequencing and data analyses* - see Adams and Schwarzbach (2011).

RESULTS AND DISCUSSION

A total evidence Bayesian tree, using ITS and the 4 cp DNAs, shows *J. microsperma* in a clade with *J. erectopatens* (Fig. 1) with 100% posterior probability. As found by Mao et al. (2010), *J. microsperma* (and with *J. erectopatens*, in this case) is in a large clade with sabinoid junipers of both the eastern and western hemispheres. The species in Figure 1 reflect the new nomenclature proposed by Adams and Schwarzbach (2012c), as listed in Table 1.

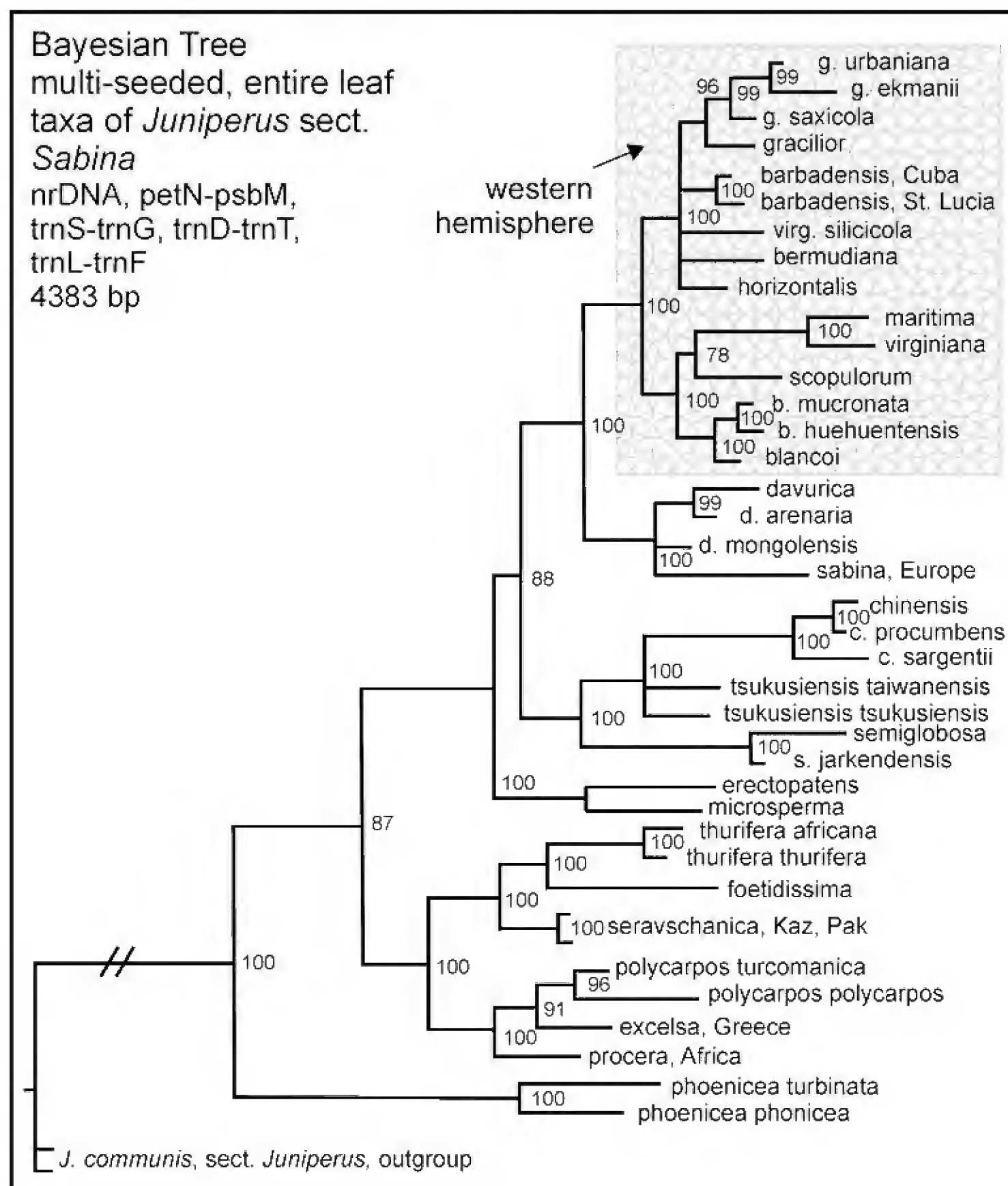


Figure 1. Bayesian tree for sect. *Sabina*, multi-seeded cone taxa. Numbers at the branch points are posterior probabilities (as percent).

Table 1. Revised nomenclature of multi-seeded, smooth leaf *Juniperus*, sect. *Sabina* (after Adams and Schwarzbach (2012c).

North America - Caribbean / Bermuda junipers

Bermuda

J. bermudiana

Caribbean

J. barbadensis

J. gracilior

J. g. var. ekmanii

J. g. var. saxicola

J. g. var. urbaniana

North American - mainland junipers

J. blancoi

J. b. v. huehuentensis

J. b. var. mucronata

J. horizontalis

J. maritima

J. scopulorum

J. virginiana

J. v. var. silicicola

Eastern hemisphere junipers

Japan, Taiwan

J. chinensis

J. c. var. procumbens

J. c. var. sargentii

J. tsukusiensis

J. t. var. taiwanensis

China, central Asia, Mediterranean

J. chinensis

J. davurica

J. davurica var. arenaria

J. davurica var. mongolensis

J. erectopatens

J. excelsa

J. foetidissima

J. microsperma

J. phoenicea

J. phoen. v. turbinata or *J. turbinata*

J. procera

J. polycarpos

J. poly. v. turcomanica

J. sabina

J. semiglobosa

J. semi. var. jarkendensis

J. seravschanica

J. thurifera

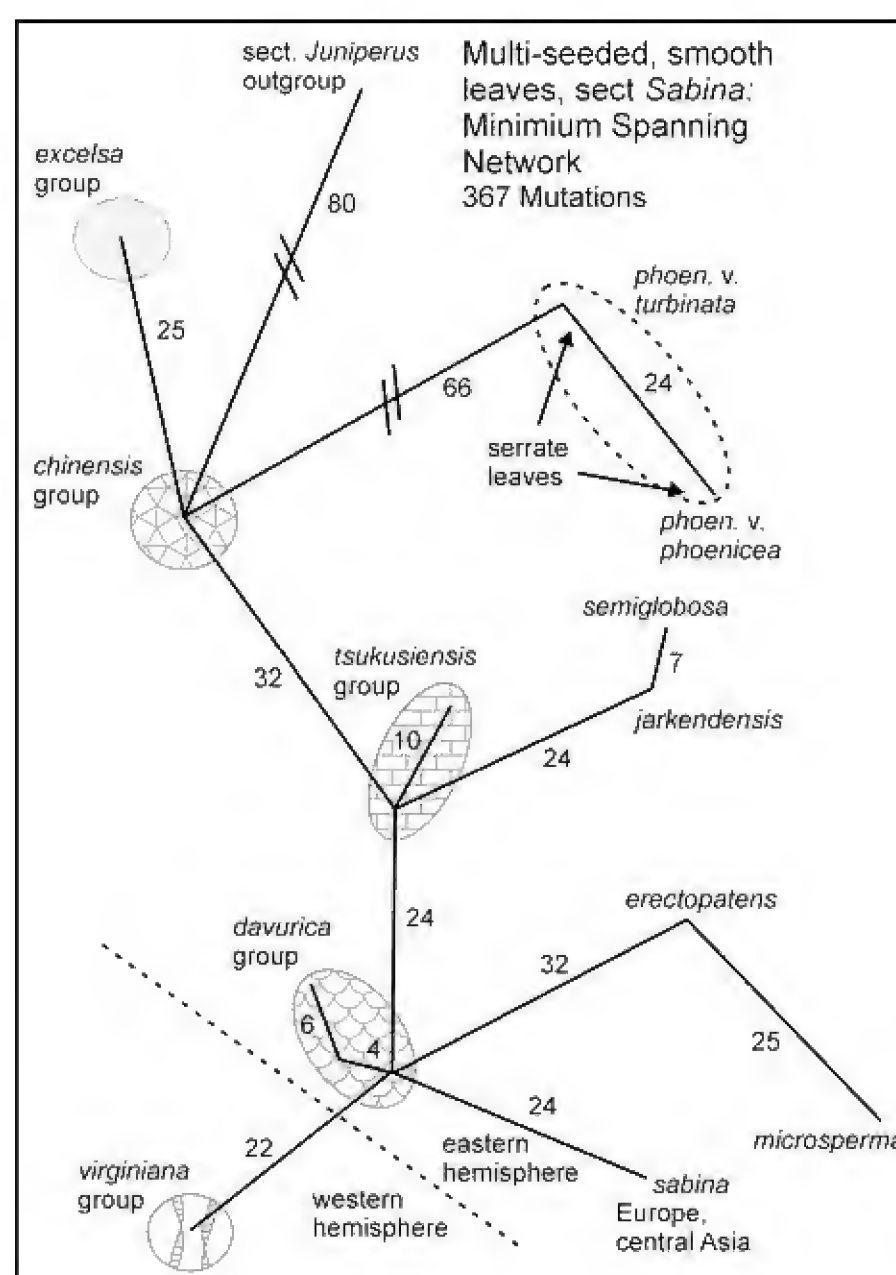
J. t. var. africana or *J. thurifera*

A minimum spanning network reveals that, although *J. microsperma* is linked to *J. erectopatens*, that linkage (25 MEs, Fig. 2, right) is very distinct and clearly in the range of other recognized species (22-32 MEs, Fig. 2, right). The second nearest link to *J. microsperma* is to *J. davurica var. mongolensis*, with a link of 38 MEs (not shown in Fig. 2, right).

Comparisons of the type specimen of *J. microsperma*, the new collection from Tibet (*Jian-Quan Liu QTP-2011-201*), and the putative *J. microsperma* from Sichuan (*Adams 8522*) revealed that *Adams 8522* is most likely a small-fruited variant of *J. saltuaria*. The reference to *J. microsperma* in Adams and Schwarzbach (2012c) should be changed to '*J. saltuaria*, variant'.

Juniperus microsperma is a poorly understood species and additional research is under way (Mao, personal comm.).

Figure 2. Minimum spanning network based on 367 mutations. Numbers next to lines are the number of MEs (mutational events).



ACKNOWLEDGEMENTS

Thanks to Kangshan Mao and Jianquan Liu for the gift of the *J. microsperma* specimen. Thanks to Tonya Yanke for lab assistance. This research supported with funds from Baylor University.

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Taxonomy of the turbinate shaped seed cone taxa of *Juniperus*, section *Sabina*: Revisited**Robert P. Adams**Biology Department, Baylor University, Box 97388, Waco, TX
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and

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ABSTRACT

The recent classification of *Juniperus microsperma* (Cheng & L. K. Fu) R. P. Adams as a member of the multi-seeded, smooth-leaved junipers in section *Sabina* (Adams and Schwarzbach, 2013), necessitates a correction in the turbinate seed cone taxa of *Juniperus*, section *Sabina*. The specimen previously identified as *J. microsperma* from Sichuan (Adams 8522-8524), appears to be a variant of *J. saltuaria* with small seed cones.

Published on-line www.phytologia.org *Phytologia* 95(2):122-124(May 1, 2013).

KEY WORDS: Taxonomy, *Juniperus*, section *Sabina*, turbinate seed cones, nrDNA, petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, *J. microsperma*.

Previously, we (Adams and Schwarzbach, 2012) reported on the taxonomy of the turbinate shaped seed cone junipers of section *Sabina*. This section has been shown to be a distinct clade (Mao et al., 2010; Adams 2011), having seed cones with elongated, pointed tips. In the previous study, a sample (Adams 8522-8524, near Zhe Gu Mtn., Maerkang County, Sichuan, China) with small seed cones thought to be *J. microsperma* (Cheng & L. K. Fu) R. P. Adams was included in the turbinate-coned junipers (Adams and Schwarzbach, 2012). However, recently, we received a specimen of *J. microsperma* from near the type locality: Song Zong, Xizang (Tibet), China, *Jian-Quan Liu QTP-2011-201*. This material proved to be quite similar to the type and is clearly most closely related to *J. erectopatens* (Cheng & L. K. Fu) R. P. Adams as determined by molecular data (Adams and Schwarzbach, 2013).

The purpose of the report is to clarify the taxonomy of the turbinate seed cone taxa of *Juniperus*, section *sabina*; in particular the placement of the mis-identified '*J. microsperma*' from Sichuan.

MATERIALS AND METHODS

See Adams and Schwarzbach (2012).

RESULTS AND DISCUSSION

The corrected Bayesian tree for the turbinate seed cone taxa of *Juniperus*, sect. *Sabina* using the nomenclature of Adams and Schwarzbach (2012) is shown in Figure 1. The tree is unchanged except for the changing of the label '*J. microsperma*' to '*saltuaria* Sichuan' (Fig. 1).

A revised minimum spanning network based on 225 mutational events (MEs) is shown in Figure 1, with the nomenclature of Adams and Schwarzbach (2012).

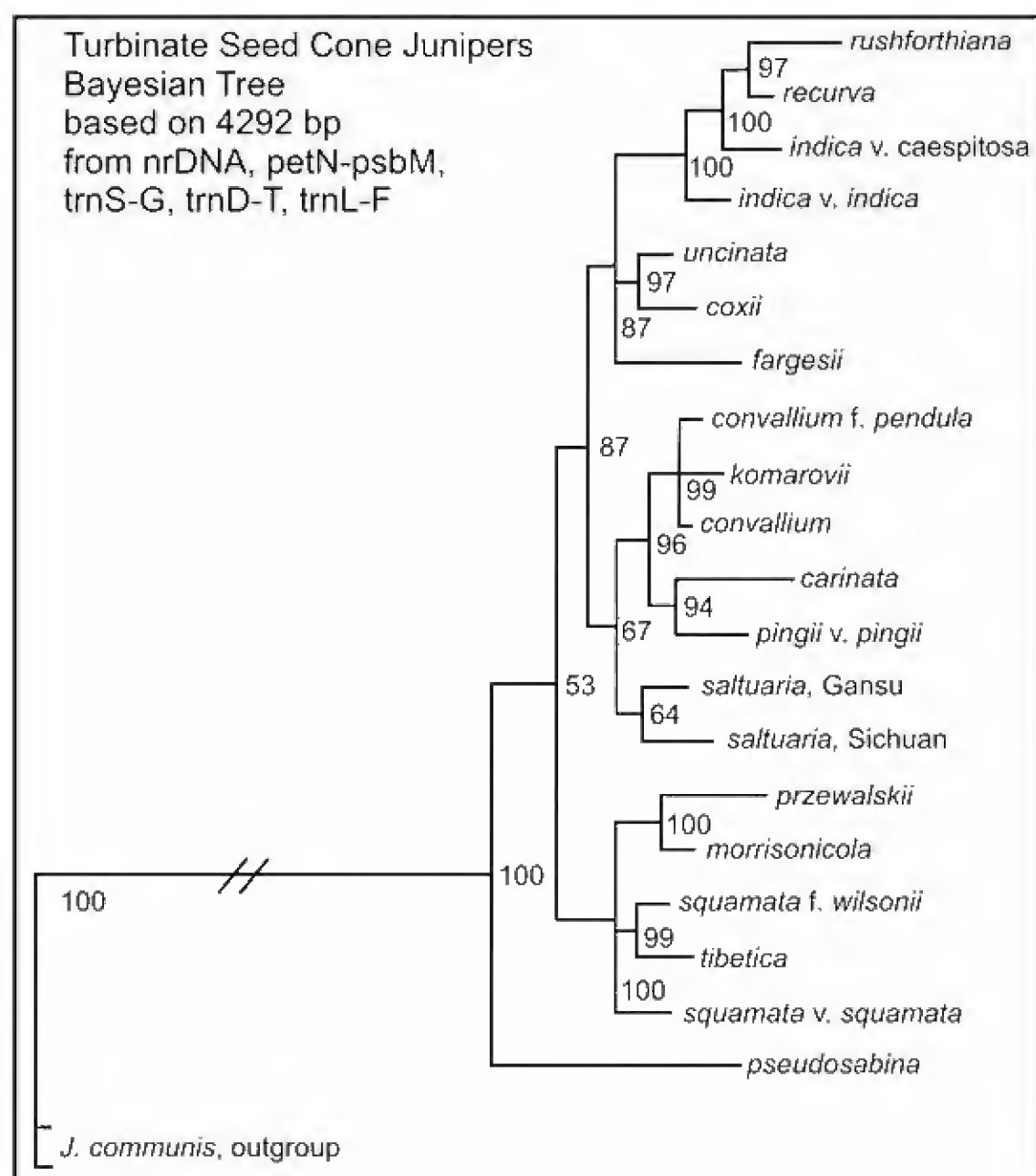


Figure 1. Corrected Bayesian tree for the turbinate seed cone taxa, sect. *Sabina*. Numbers at the branch points are posterior probabilities (as percent). Nomenclature follows Adams and Schwarzbach (2012).

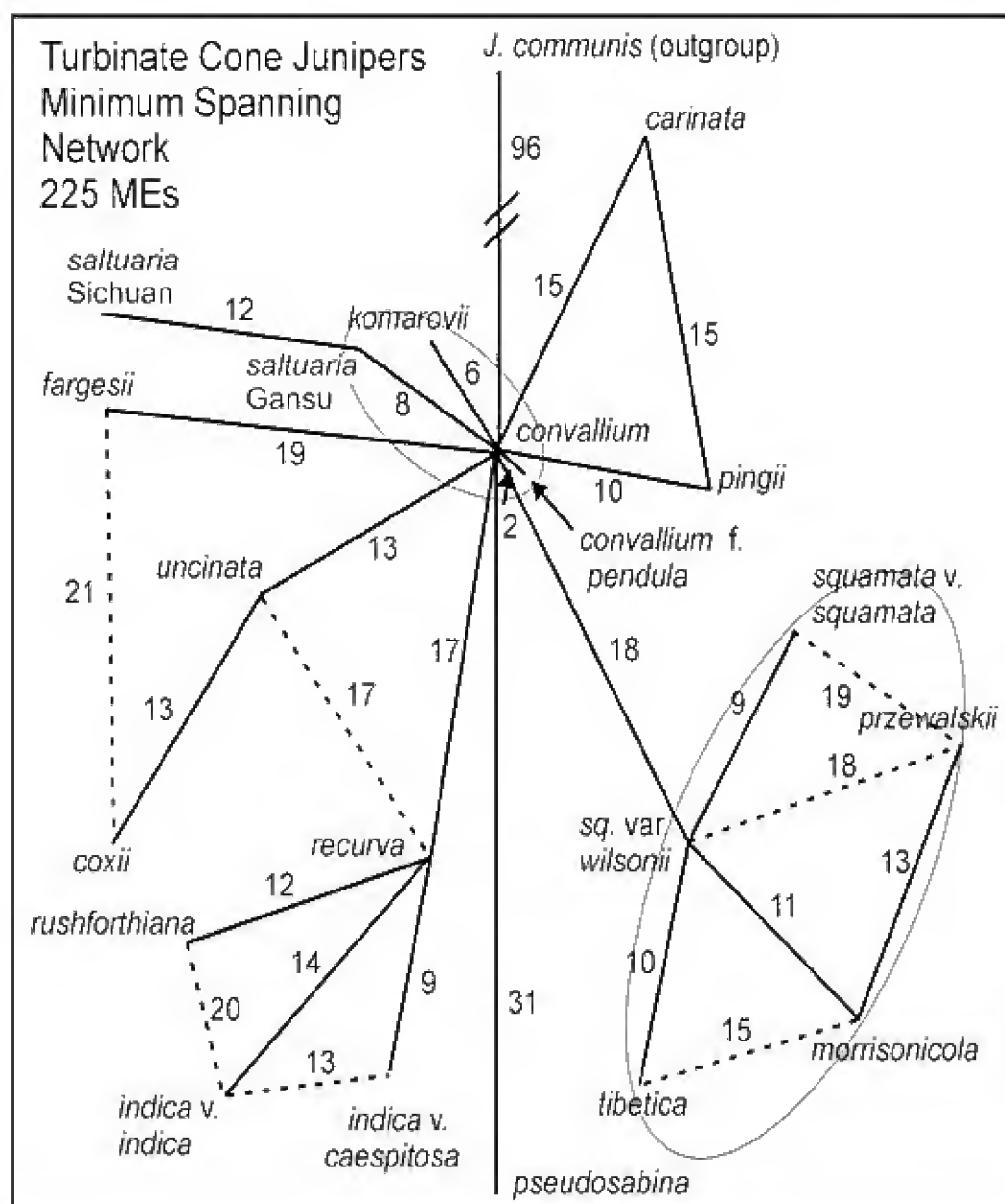


Figure 2. Corrected minimum spanning network based on 225 MEs. Numbers at branch points are the number of mutational events (MEs). Nomenclature follows Adams and Schwarzbach (2012).

A summary of the previous nomenclature and currently supported nomenclature in this section is presented in Table 1.

Table 1. Comparison of Adams and Farjon taxonomic treatments of taxa in this study. Taxa with DNA sequencing support for a modified taxonomic status are in bold.

Adams(2011)	Farjon (2005, 2010)	Supported in Adams and Schwarzbach (2012) and used in this paper
<i>J. convallium</i>	<i>J. convallium</i>	<i>J. convallium</i>
<i>J. coxii</i>	<i>J. recurva</i> v. <i>coxii</i>	<i>J. coxii</i>
<i>J. indica</i>	<i>J. indica</i>	<i>J. indica</i>
<i>J. i. var. caespitosa</i>	<i>J. i. var. caespitosa</i>	<i>J. indica</i> var. <i>caespitosa</i> ?
<i>J. i var. rushforthiana</i>	<i>J. indica</i>	<i>J. rushforthiana</i>
<i>J. komarovii</i>	<i>J. komarovii</i>	<i>J. komarovii</i>
<i>J. morrisonicola</i>	<i>J. squamata</i>	<i>J. morrisonicola</i>
<i>J. pingii</i>	<i>J. pingii</i>	<i>J. pingii</i>
<i>J. p. var. carinata</i>	<i>J. p. var. wilsonii</i>	<i>J. carinata</i>
<i>J. przewalskii</i>	<i>J. przewalskii</i>	<i>J. przewalskii</i>
<i>J. p. f. pendula</i>	<i>J. przewalskii</i>	<i>J. convallium</i> f. <i>pendula</i>
<i>J. pseudosabina</i>	<i>J. pseudosabina</i>	<i>J. pseudosabina</i>
<i>J. recurva</i>	<i>J. recurva</i>	<i>J. recurva</i>
<i>J. r. var. uncinata</i>	<i>J. recurva</i> ?	<i>J. uncinata</i>
<i>J. saltuaria</i>	<i>J. saltuaria</i>	<i>J. saltuaria</i>
<i>J. squamata</i>	<i>J. squamata</i>	<i>J. squamata</i>
<i>J. s. var. fargesii</i>	<i>J. squamata</i>	<i>J. fargesii</i>
<i>J. s. f. wilsonii</i>	<i>J. pingii</i> f. <i>wilsonii</i>	<i>J. s. var. wilsonii</i>
<i>J. tibetica</i>	<i>J. tibetica</i>	<i>J. tibetica</i>

There are unresolved taxonomic problems in *J. squamata*, *J. squamata* var. *wilsonii*, *J. fargesii*, and other specimens with decurrent leaves. The problems are under additional study.

ACKNOWLEDGEMENTS

This research was supported in part by funds from NSF projects DEB-0316685 to RPA and AES as well as DEB-0629402 to AES and funds from Baylor University (RPA).

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Analysis of decurrent-leaved Himalayan junipers: Discordance between leaf morphology and DNA

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ABSTRACT

Several accessions of decurrent-leaved junipers of the turbinate seed cone taxa of *Juniperus* section were DNA bar-coded using data from nrDNA, and 4 cp regions in an attempt to identify unusual specimens of *J. coxii*, *J. fargesii*, *J. squamata*, and *J. recurva* from both wild and cultivated sources. The variation in the size, shape and branching angles of decurrent leaves was incongruent with the DNA bar-coding data. Specimens from these taxa differ chiefly in leaf morphology. The plasticity of decurrent leaves, neoteny and hybridization appear to make the use of only leaf type data unreliable for identification of these questionable or difficult specimens. Published on-line: www.phytologia.org *Phytologia* 95(2):125-131 (May 1, 2013).

KEY WORDS: *Juniperus*, Cupressaceae, DNA bar-coding, decurrent leaves.

The turbinate seed cone *Juniperus*, section *Sabina* contain a group of closely related taxa with decurrent leaves (Adams and Schwarzbach, 2012, 2013). *Juniperus* has at least three types of leaves: acicular (as found in spruce, fir, etc.) where the entire leaf drops from the stem (Fig. 1, left); decurrent (whip) leaves with a sheath that clasps the stem and a blade that extends outward from the blade at various angles (Fig. 1, center); and scale leaves with tips that are usually appressed to the next scale leaf on the stem (Fig. 1, right). In section *Sabina*, decurrent leaves are always present on a seedling and the plant continues to make these juvenile (decurrent) leaves for several years. Most junipers in sect. *Sabina* begin the production of scale-like leaves (adult leaves) after a few years and then decurrent (whip) leaves are only found at the tips of rapidly growing branches.

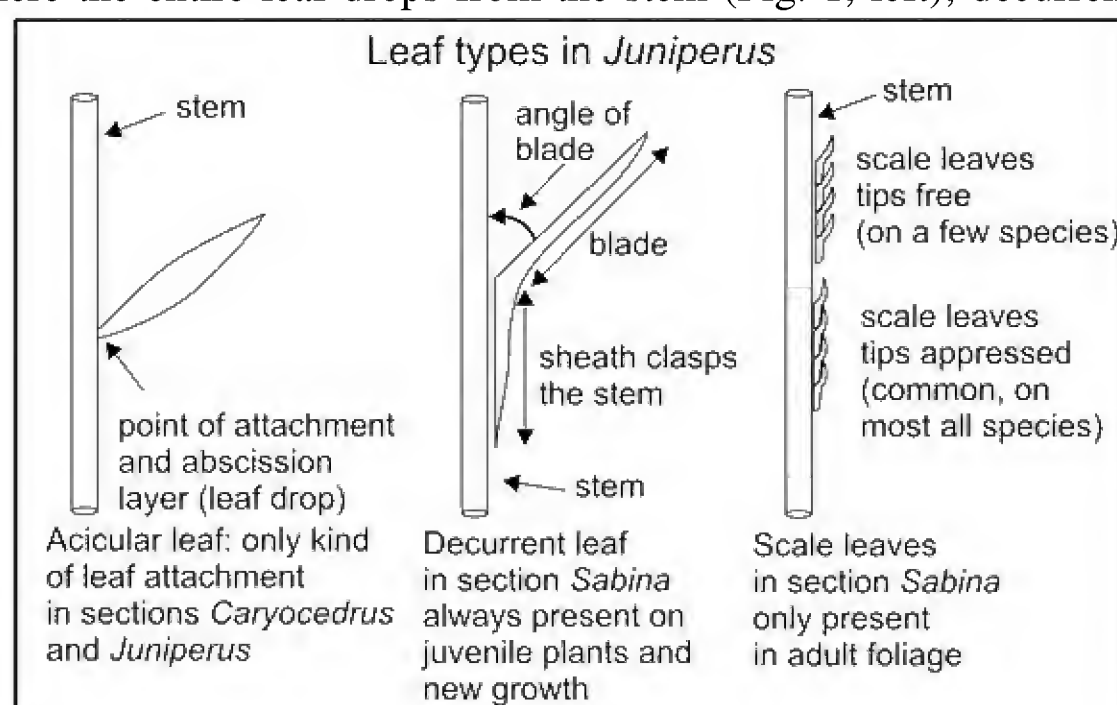


Figure 1. Leaf types in *Juniperus*.

However, for nearly every species of sect. *Sabina*, the senior author has observed a few mature (adult) trees in a population that are frozen in neoteny that have only juvenile (decurrent or whip) leaves. *Juniperus chinensis* is very unusual in that it has both decurrent and scale-like leaves interspersed on branches in mature (adult) trees. *Juniperus saxicola* (now recognized as *J. gracilior* var. *saxicola* Britt. & Wils.) is a taxon that is frozen in neoteny and has only juvenile (decurrent) leaves. Another Caribbean juniper (*J. barbadensis* var. *barbadensis*) has several mature (adult) trees in the little population at the summit of Petit Piton, St. Lucia, BWI that have only decurrent (juvenile) leaves (Adams, 2011).

It appears that several taxa in the turbinate group also exhibit neoteny (Fig. 2, *J. squamata*, *J. s.* var. *wilsonii*, *J. fargesii*, *J. morrisonicola*, *J. recurva* and *J. coxii*). Although these taxa appear distinct in the photos in Figure 2, in nature and cultivation, there is tremendous variation in size, shape and blade angle of their leaves. This has presented considerable difficulty in the identification of specimens from these taxa.

The purpose of this paper is to report on DNA bar-code analysis of ‘difficult’ specimens that have been tentatively identified to determine if DNA bar-coding could aid in classifying these ‘difficult’ specimens.

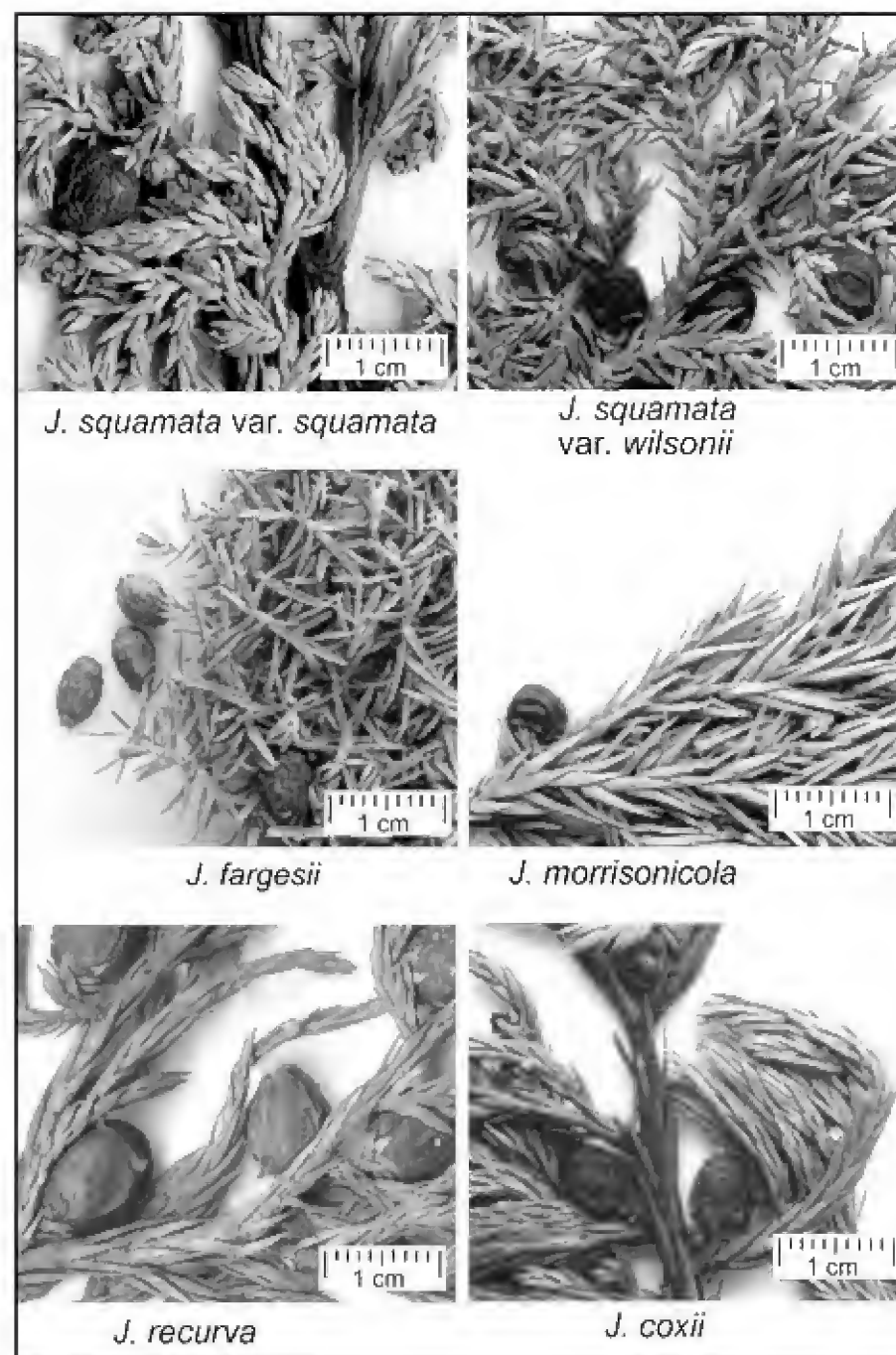


Figure 2. Leaves and seed cones of selected *Juniperus* species.

MATERIALS AND METHODS

Specimens used in this study: *J. carinata*, Adams 8497-99, near White Horse Mtn., Deqin County, Yunnan, China, *J. convallium*, Adams 6781-83, 17 km e Tewo, Gansu, China, *J. convallium* f. *pendula*, Adams 6779, Langmusi, Gansu, China, *J. communis* L. var. *communis*, Adams 7846, 7847, Stockholm, Sweden (outgroup), *J. coxii*, Adams 8137-38, clone from Type tree, Abbotsmarsh Arboretum, UK, ex Burma, Chimili valley, *J. fargesii*, Adams 6769-6770, Lian Wha Mtn., s of Kangle, Gansu, China, *J. indica*, Adams 8775, Dumpa, Jomson, Nepal, *L N Sharma J2, J16*, Dhikur Pohkari, Manang, Nepal, *J. indica* var. *caespitosa*, Adams 7625-27, near Yangjin Gompa, Nepal, *J. komarovii*, Adams 8518-20, near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. microsperma*, Adams 8522-24, near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. morrisonicola*, Adams 8681-2, Younger Bot. Gard., Scotland, ex Taiwan, *J. pingii*, Adams 8506-7, near White Horse Mtn., Deqin County, Yunnan, China, *J. pingii* var. *miehei*, Boufford, Kelley, Ree & Wu 29745, (acc. 13598) Baxoi Xian, Anjiu La (pass), Xizang (Tibet), China, *J. pseudosabina*, Adams 7808-10, 30 km n Jarkent (Paniflor), Kazakhstan, *J. przewalskii*, Adams 6775-77, 25 km w Jone, Gansu, China, *J. recurva*, Adams 7215, 7219, Cholan Pati lodge, Nepal, *L N Sharma GJ14, GJ15*, (acc. 12140, 12141), Mobro Madi, Nepal, *J. rushforthiana*, Adams 8140-41, Abbotsmarsh Arboretum, UK, ex Lingshi, Bhutan *J. saltuaria*, Adams 6789-91, on Duoer River, 23 km e Forestry Station, Gansu, China, *J. squamata*, Adams 6796, Xian Bot. Garden, Shaanxi, China, *J. squamata* cv. *Meyeri*, Adams 13547, Arnold Arboretum acc. 10316*A, *J. tibetica*, Adams 8512-16, on

Maerkan River, near Zhe Gu Mtn., Maerkan County, Sichuan, China, *J. uncinata*, Adams 7212-14, Lauri Binayak, Nepal.

Specimens of uncertain affinity:

F3, F4, 'fargesii', Adams 8491-93, near White Horse Mtn., Zhongdian County, Yunnan, China,

F6, 'fargesii', K. Rushforth 3704, (acc. 13505) Nyima La, descent into Rong Valley at Tumbatse, Tibet, cult. in UK,

F7, 'fargesii', Chadwell 6137, , (acc. 13506) Nyima, Annapurna Himalayas, Nepal, cult. in UK,

F8, 'fargesii', K. Rushforth 8258, (acc. 13507) on s side of Jira La, Arunachal Pradesh state, India, cult. in UK,

P2, 'pingii', D. Boufford 37031, (acc. 11767), seeds, Sichuan, China,

S3, 'squamata', K. Rushforth 5405A, (acc. 8287), Nyima La, near 47 campsite, Xizang (Tibet), China,

S4, S5, 'squamata', L N Sharma J2GJ10, GJ11, (acc. 12936, 12937), Nyarku, Nepal,

S6, 'squamata', Adams 7012, Kunming Botanic Garden, Kunming, China,

S7, 'squamata', K. Rushforth 907, (acc. 13504), on route from Linghsi Dzong to Yale La, Bhutan, cult. in UK,

U3, 'uncinata', K. Rushforth 9512, (acc. 13508), near Jang Gomba campsite, India, cult. in UK,

W1, '*J. squamata* f. *wilsonii*', Adams 5521, Accession 1010-64A, cultivated from seeds from E. H. Wilson 985 (Holotype) collection, Arnold Arboretum, USA, ex. China,

W4, 'wilsonii or 'pingii', K. Rushforth 3853, (acc. 12912), n of Lhasa, Xizang (Tibet), China, cult. in UK,

Voucher specimens are deposited in the herbarium, BAYLU, Baylor University.

DNA extraction, PCR amplification, sequencing and data analyses

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. 5.4 (Drummond et al. 2011), the MAFFT alignment program and the PAUP* program, version 4.0b10 (Swofford 2003) for neighbor joining, parsimony, and maximum likelihood tree searches. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975).

RESULTS AND DISCUSSION

The overall pattern of variation (Fig. 3) is the same as shown by Adams and Schwarzbach, 2012, 2013). Our points of interest are in the groupings that contain the ‘difficult’ specimens. Specimens collected as ‘fargesii’, with leaf blades diverging from the stem by 45° - 90° are scattered in: Group I (F3,

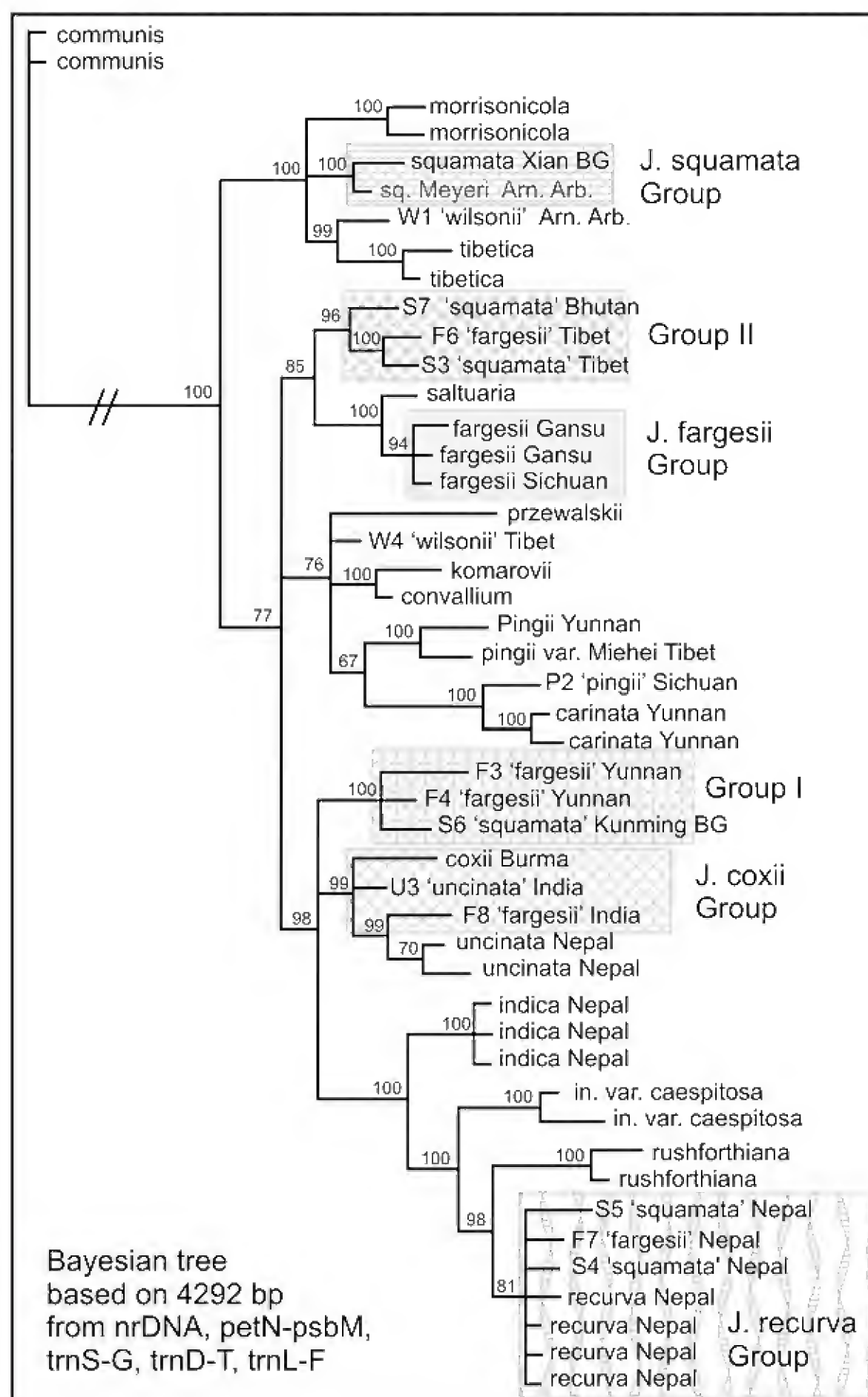


Figure 3. Bayesian tree showing the ‘difficult’ specimens in relation to other members of the turbinate seed-cone taxa of sect. *Sabina*. The divergent species, *J. pseudosabina* is not shown. Numbers at the branch points are posterior probabilities (as percent). Groups are discussed in the text.

F4), Group II (F6) *J. coxii* Group (F8) and *J. recurva* Group (F7). Specimens referred to as ‘squamata’, with leaf blades mostly appressed are found in: Group I (S6), Group II (S3, S7) and *J. recurva* Group (S4, S5). The two specimens of ‘wilsonii’ are in a clade with *J. tibetica* (W1) and in a clade with *J. pingii*, *J. komarovii*, etc. (W4).

Utilizing the large amount of indel information as well as substitutions gives a minimum spanning network with a slightly different perspective (Fig. 4).

Juniperus fargesii group

Three of the *J. fargesii* individuals (*Adams 6769, 6770, Gansu; Adams 8521, Sichuan*) displayed no variation in their DNA bar-codes.

Notice the variation in blade angle from nearly 90° in the Gansu specimens to about 45° in the Sichuan specimen (Fig. 5).

Several other ‘difficult’ specimens collected as ‘fargesii’, are scattered about the network: F3, F4 is in Group I; F8 ‘fargesii’ India is in the *J. coxii* group; F6 ‘fargesii’ Tibet is in Group II (Fig. 4); and F7 ‘fargesii’ Nepal is in the *J. recurva* group.

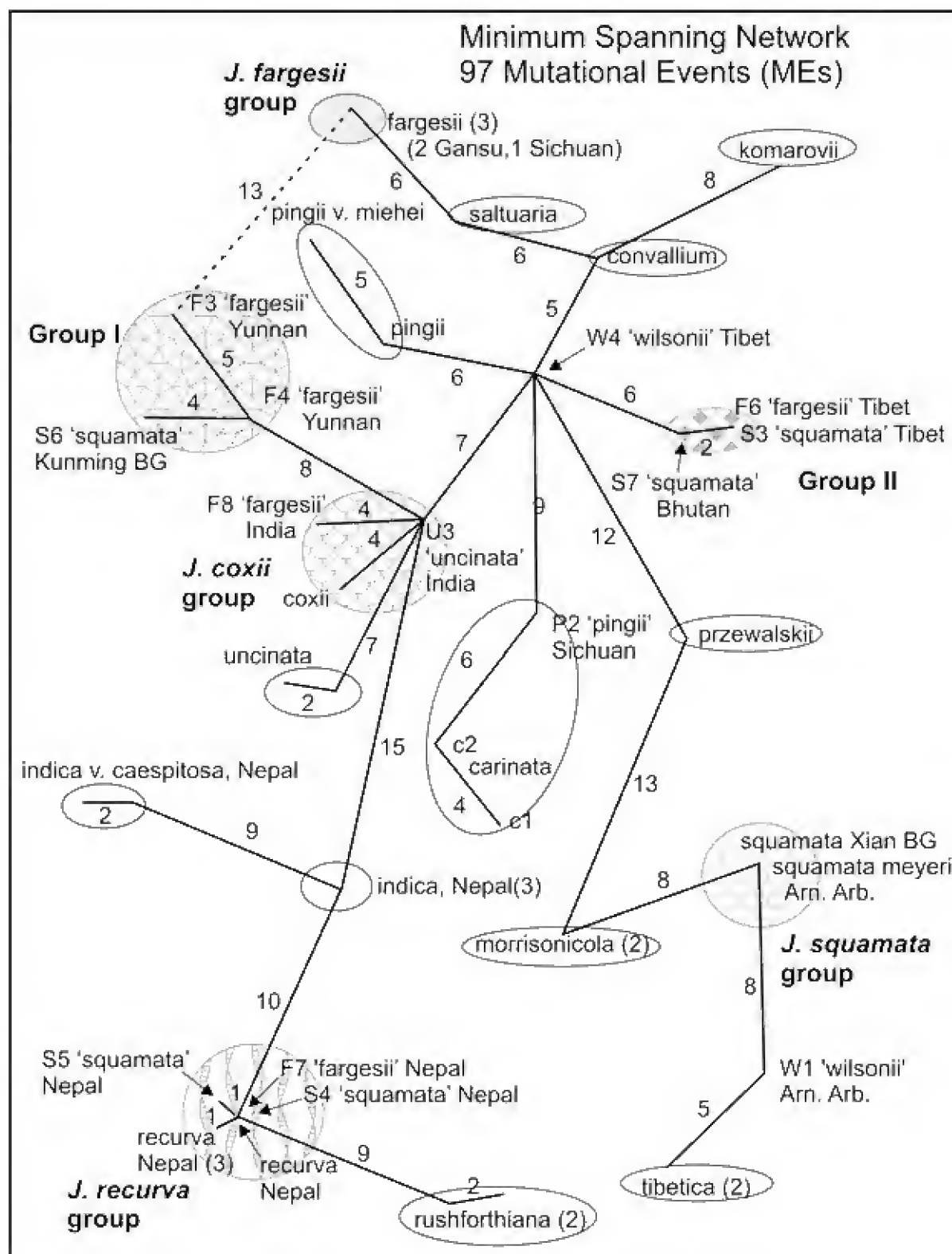


Figure 4. Minimum spanning network based on 97 MEs. Numbers on lines are the number of MEs. Open ovals enclose currently recognized species and varieties.



Figure 5. *J. fargesii* group: Gansu, 6769 and 6770; Sichuan 8521.

***Juniperus coxii* group:** *J. coxii*, Yunnan, 8508, F8 ‘fargesii’ India, KR8258, and U3 ‘uncinata’ India, KR9512,

This group is linked to *J. coxii*, Yunnan by ‘uncinata’ KR9512, India (4 MEs, Fig. 3) and ‘fargesii’ KR8258, India (4 MEs, Fig. 3.). *Juniperus coxii* has only decurrent leaves with the blades tightly appressed to the stem (Fig. 6, left). The photos of both ‘fargesii’ KR8258 and ‘uncinata’ KR9512 are from plants cultivated in the UK. Their foliage may be still juvenile.

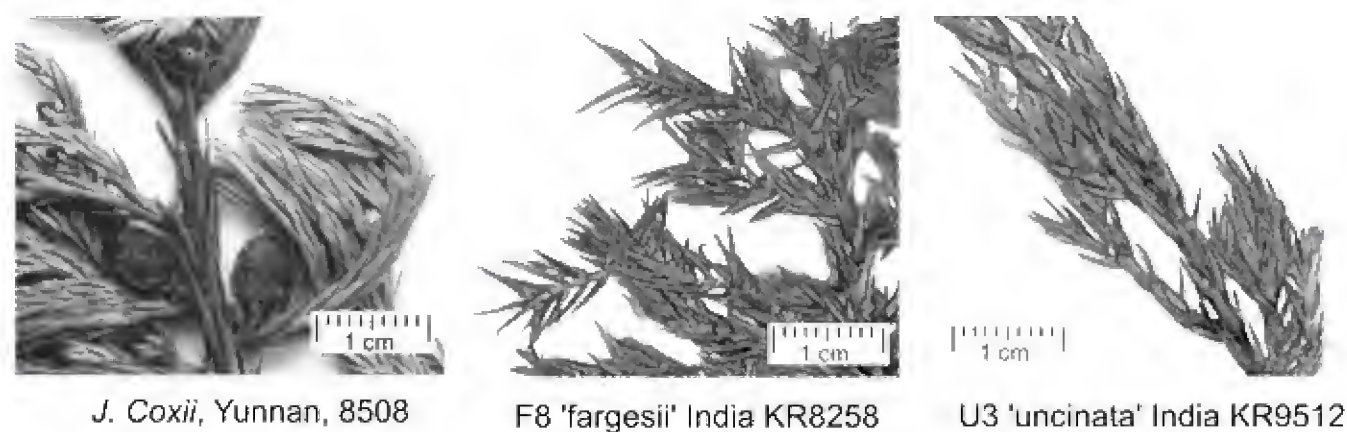


Figure 6. ***J. coxii* group:** *J. coxii*, Yunnan; F8 ‘fargesii’ India, KR8258, cultivated in UK; and U3 ‘uncinata’ India, KR9512, cultivated in UK.

***Juniperus recurva* group:** *J. recurva*, Nepal 7219, S5 ‘squamata’ Nepal 12937; and F7 ‘fargesii’ Nepal, Chadwell 6137.

Juniperus recurva has decurrent leaves with the blades tightly appressed. However, ‘squamata’ Nepal Sharma 12937; and ‘fargesii’ Chadwell 6137, Nepal differed by only 1 or 2 MEs from the typical *J. recurva*. Yet, their leaves are quite different from *J. recurva* (Fig. 7). The photo of ‘fargesii’ Chadwell 6137, Nepal (Fig. 7) is from cultivated material and the foliage may be juvenile.



Figure 7. ***J. recurva* Group:** *J. recurva*, Nepal 7219, S5 ‘squamata’ Nepal 12937; F7 ‘fargesii’ Nepal, Chadwell 6137, cultivated in UK.

***Juniperus squamata* Group:** *J. squamata* Xian Bot. Gard. 6795 and *J. squamata* cv. *Meyeri*, Arn. Arb, 13547.

This group contains two specimens that had no DNA differences (*J. squamata*, Xian Bot. Gard., 6795, *J. squamata* var. *Meyeri*, Arn. Arb., 13547). These specimens are very similar morphologically (Fig. 8) and are similar to the type. These *J. squamata* specimens are linked by a difference of 8 ME to *J. morrisonicola* and W1 ‘wilsonii’. W1 ‘wilsonii’ from Arnold Arboretum looks very much like the type specimen, however, it differs by only 5 MEs from *J. tibetica* (shown with adult leaves in Fig. 8, right-most). It is surprising that none of the putative *J. squamata* in this study had DNA like *J. squamata* from Xian Botanic Garden.

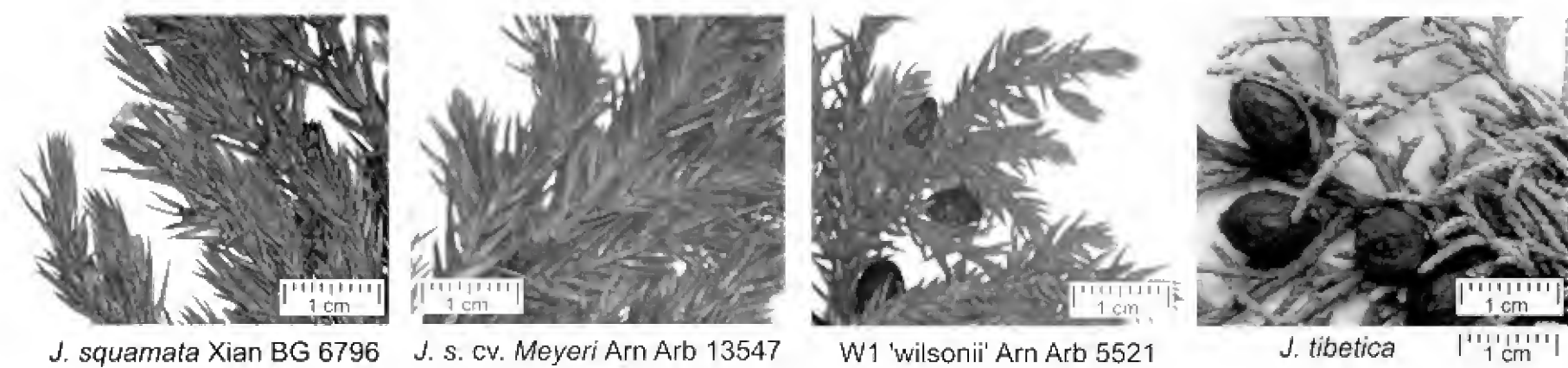


Figure 8. *J. squamata* Group: *J. squamata*, Xian Bot. Gard.; s. cv. *Meyeri*, Arn. Arb.; plus W1 '*wilsonii*', Arn. Arb. 5521 and *J. tibetica* that differs by only 5 MEs from W1 (see Fig. 4).

Group I: F3 'fargesii' Yunnan, 8491, F4 'fargesii' Yunnan, 8492 and S6 'squamata' cultivated at Kunming Botanic Garden, 7012.

Group I is not very uniform in its DNA (Fig. 3), nor in its morphology. The two 'fargesii' from Yunnan have large blade angles (Fig. 9) that would seem typical of *J. fargesii* (Fig. 4), but note that 8491 is separated by 13 MEs from typical *J. fargesii* (6769, Fig. 3). The cultivated 'squamata' 7012 from KBG is only 4 MEs from 'fargesii' 8492 (Fig. 3), and its blade tips are quite appressed (Fig. 9).



Figure 9. Group I: F3 'fargesii' Yunnan 8491, F4 'fargesii', Yunnan 8492 and S6 'squamata', cultivated at Kunming Botanic Garden, 7012

Group II. F6 'fargesii', Tibet, KR3704, S7 'squamata', Bhutan, KR907 and S3 'squamata', Tibet, KR5405A.

The DNA of this group is most like that of *J. convallium* (Fig. 3). Group II is diverse in both DNA and morphology (Fig. 10). However, photos of specimens of F6 'fargesii', KR3704, Tibet and S7 'squamata', Bhutan, KR907 are from materials cultivated in the UK and may have juvenile leaves.

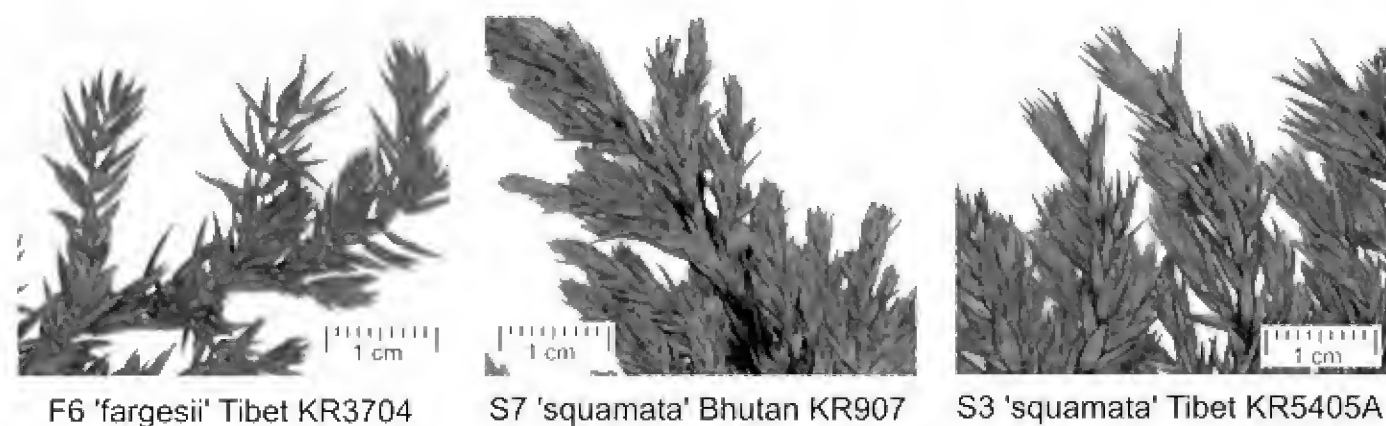


Figure 10. **Group II:** F6 'fargesii', Tibet KR3704, cultivated in UK, S7 'squamata', Bhutan, KR907, cultivated in UK and S3 'squamata', Tibet KR5405A.

The presence of Groups I and II, not clearly allied with any known species suggests that these may be cryptic species that have not previously been recognized, or problems of classifying these 'difficult' specimens may be a result of incomplete lineage sorting. Degnan and Rosenberg (2009) defined incomplete lineage sorting as “failure of two of more lineages in a population to coalesce, leading to the possibility that at least one of the lineages first coalesces with a lineage from a less closely related population”. Yu et al. (2011, 2012) recently addressed the problems of extensive hybridization in nature and incomplete lineage sorting, and their effects on phylogenetic trees and networks.

Adams et al. (2009) found hybridization between *J. recurva* and *J. uncinata* in Nepal. It also seems very likely that some of the ‘difficult’ specimens analyzed in this study are hybrids or backcrosses. This could be a major limitation in applying DNA bar-codes to identify ‘difficult’ specimens with our current numerical methods. This is particularly true in this (and many DNA studies) where most of the data is from cp DNA that is uni-parentally inherited.

Overall, this exercise in DNA bar-coding these taxa left much to be desired. The discordance of bar-codes and morphology renders the study of little practical use. Field identification was poorly correlated with assignment to species by DNA bar-coding. The plasticity of decurrent leaves, neoteny and hybridization appear to make the use of only leaf type data as unreliable for identification of these taxa.

ACKNOWLEDGEMENTS

Thanks to Tonnie Yanke for lab assistance and to Lila Nath Sharma for collecting specimens in Nepal. Thanks to Arnold Arboretum, Kunming Botanic Garden and Xian Botanic Garden for providing specimens. This research was supported in part by funds from Baylor University (RPA).

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Chemosystematics of *Juniperus*: Effects of leaf drying on the essential oil composition of *Juniperus pinchotii*, changes during the first 48 hours of drying

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ABSTRACT

A bulk collection of terminal branchlets was made from *J. pinchotii* and subjected to drying at 42-45° C for up to 48 hrs. The oils were distilled and analyzed from fresh leaves and those dried for 4h, 8h, 12h, 24h and 48h. Sabinene, α -terpinene, cis-sabinene hydrate, camphor, citronellol and bornyl acetate declined during drying, whereas borneol increased. The largest changes occurred between 24 and 48 h of drying (as the leaves became brittle), which explains the previous report of changes in oils between fresh and 0.5 mo. (Adams 2013).

Published on-line: www.phytologia.org *Phytologia* 95(2): 132-137 (May 1, 2013).

KEY WORDS: *Juniperus pinchotii*, oils from dried leaves, chemosystematics, terpene decomposition.

Recently, I reported (Adams, 2013) on changes in volatile leaf oil composition from *Juniperus pinchotii* leaves air dried (42-45°C, 24h) and stored at room temperature (22° C, RT) for up to 24 mos. The oil yields showed a slight decline initially, but remained fairly constant (Fig. 1). Camphor, camphene hydrate and citronellal declined (mg/g dry foliage) in fresh vs. 0.5 mo. samples. Borneol increased during storage (on a mg/g basis). This may be due to the loss of acetate by bornyl acetate and/or oxidation of terpenes to produce borneol.

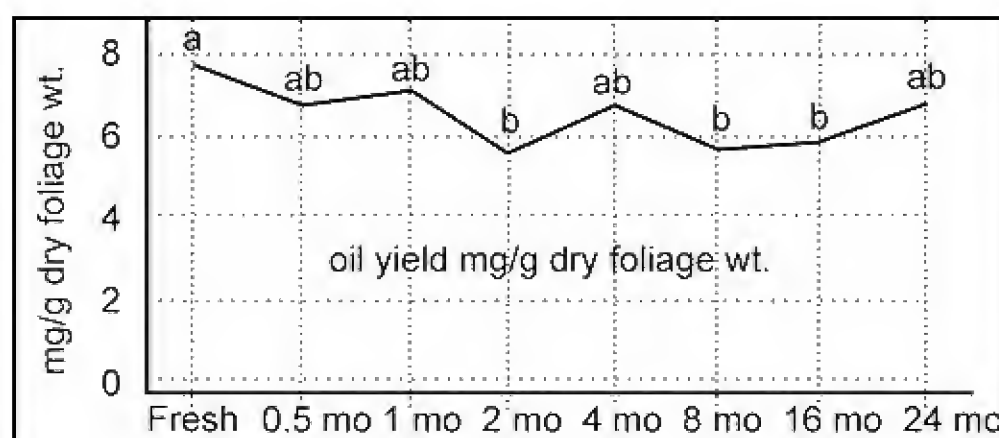


Figure 1. Variation in oil yield (mg/g) from specimens stored at RT for up to 24 mo. Samples sharing a common letter are not statistically different (P=0.05). (from Adams, 2013).

The major trend (Adams, 2013) was the decrease in camphor, camphene hydrate, and citronellal during drying (fresh vs. 0.5 mo., Fig. 2). In addition, there was a decrease in bornyl acetate and an increase in borneol during the 24 mo. storage at RT. Overall, most of the changes occurred during the drying process, ie. fresh and 0.5 mo. samples. Because the purpose of that study was to examine oil stability *in situ* over a long period (24 mo.), no detailed information was gathered concerning oil changes during the drying process (0 - 48h).

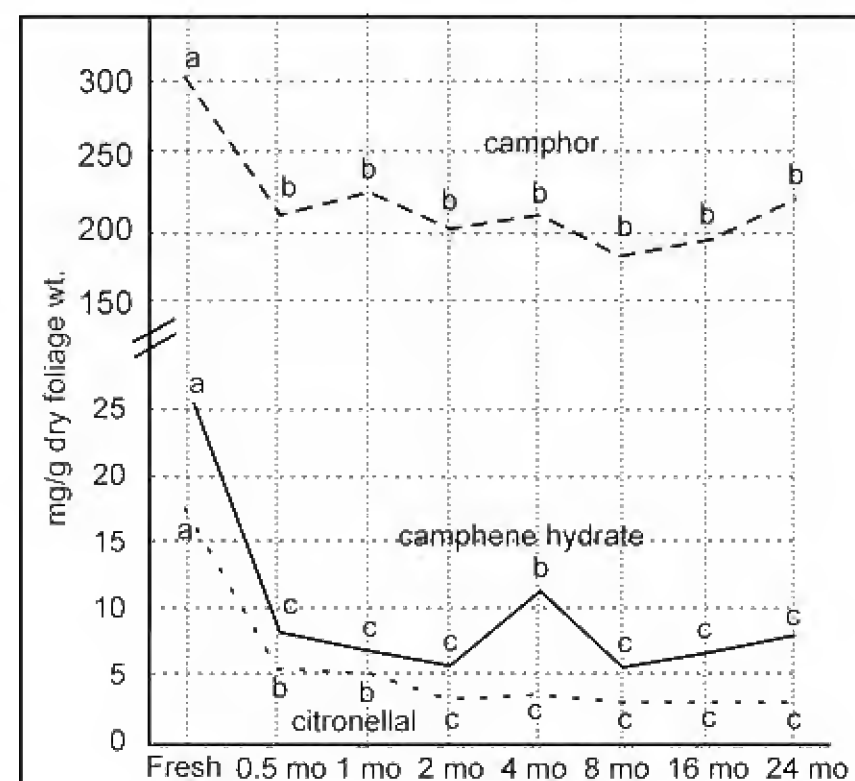


Figure 2. Variation in camphor, camphene hydrate and citronellal

The purpose of this study is to report on the initial changes in composition of leaf oil from *J. pinchotii* leaves during the first 48 hr of drying at 42-45°C. The literature was recently reviewed (Adams, 2013).

MATERIALS AND METHODS

Plant material - *J. pinchotii*, Adams 13742, 14.2 mi. s of Claude on Tex 209, Armstrong Co., TX. Voucher specimen is deposited in the Herbarium, Baylor University (BAYLU).

Isolation of oils - Fresh (200 g) and air dried (100 g) leaves were co-steam distilled with 2 mg of methyl decanoate (internal standard) for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields.

Analyses - The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. For the comparison of oils obtained from leaves stored for various periods, associational measures were computed using absolute compound value differences (Manhattan metric), divided by the maximum observed value for that compound over all taxa (= Gower metric, Gower, 1971; Adams, 1975). Principal coordinate analysis was performed by factoring the associational matrix based on the formulation of Gower (1966) and Veldman (1967). Principal component analysis (PCA) follows Veldman (1967).

RESULTS AND DISCUSSION

Comparisons of the leaf components (on a mg/g foliage oven dry weight basis) from the leaves of *J. pinchotii* from fresh vs. air dried (42 - 45° C) for different periods are shown in Table 1. There is a significant drop in oil yield between 24 and 48h (Table 1, Fig. 3). It should be noted that the plant press was opened to remove a newspaper with pressed leaves at each sampling interval. At 24 h, some of the leaves were still obviously not dried enough for storage (i. e. brittle). After 48 h all the leaves were brittle (i.e., break when bent). It appears that this is the point where most chemical changes occur. It should be noted that this is also the point where one would remove the specimens for herbarium storage. Drying for less time would be insufficient to prevent mold growth on specimens. The increase in oil after 8 h (Fig. 3) might be due to the hydrolysis of terpene glycosides making some terpenes more available for removal by steam distillation.

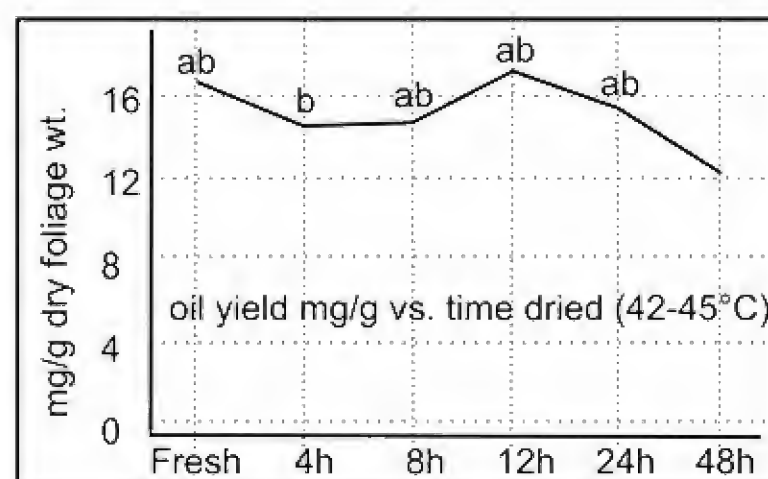


Figure 3. Oil yield vs. time drying. Data points with a common letter are not significantly different by SNK range tests.

Several of the volatile leaf oil components exhibit highly significant declines during drying: sabinene, α -terpinene, cis-sabinene hydrate, camphor citronellol and bornyl acetate (Table 1). These components exhibited a decline between fresh and 4 h, an increase from 8 to 12 h, then a decline from 12 to 48 h, with the major decline between 24 and 48 h when the leaves become brittle (Fig. 4). Camphor and sabinene (the major oil components, accounting for about 80% of the leaf oil) have a very similar pattern (Fig. 4). It seems likely that the overall loss of oil (see Fig. 3) is a factor in the decline of these two major components.

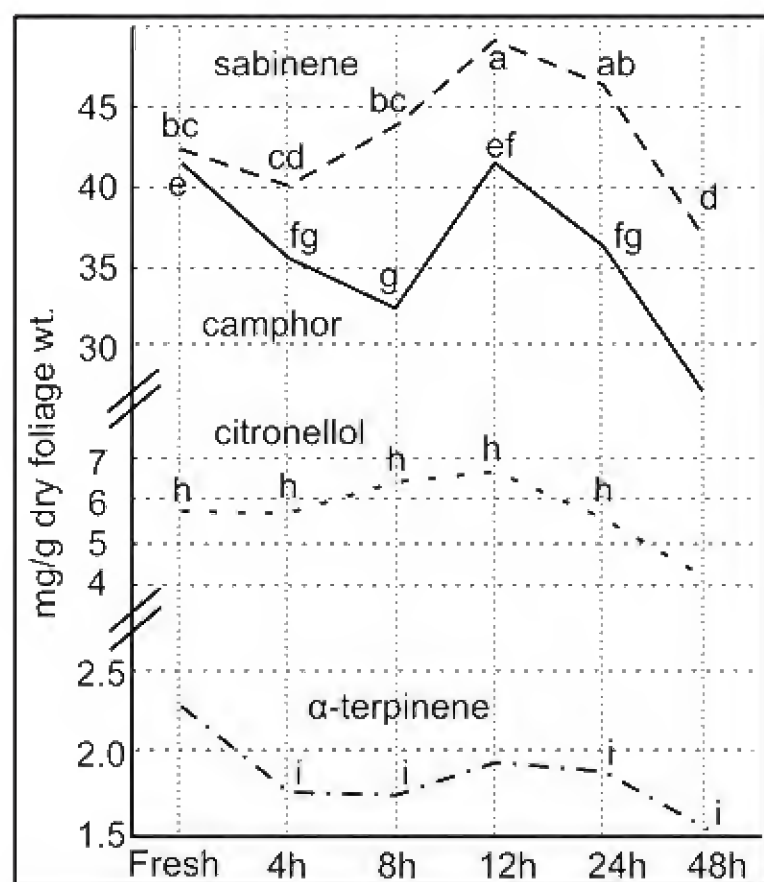


Figure 4. Changes in sabinene, camphor, citronellol and α -terpinene during drying.

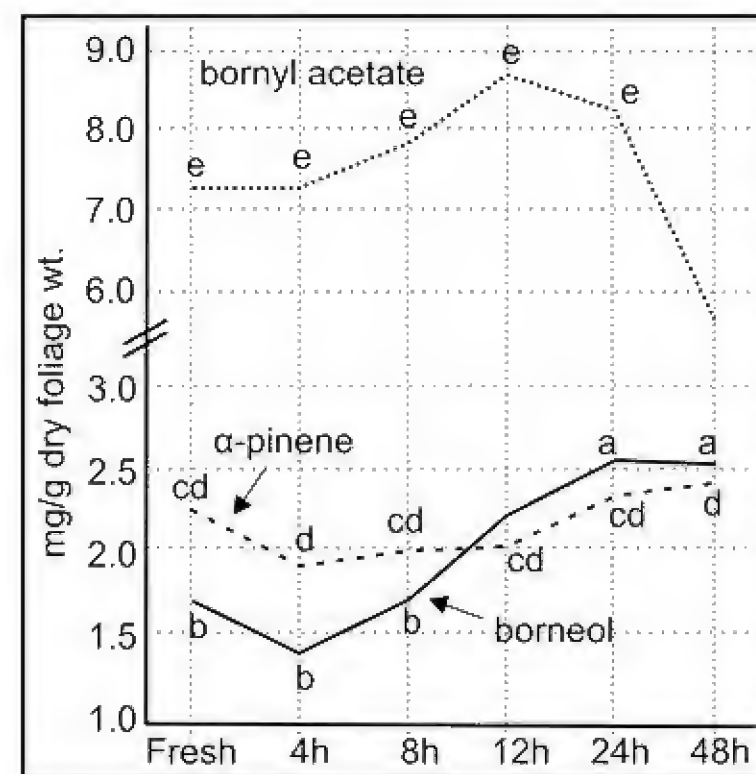


Figure 5. Changes in bornyl acetate, borneol and α -pinene during drying.

Borneol and to a lesser extent, α -pinene, increased with drying (Fig. 5). Notice that if bornyl acetate were de-acetylated to produce borneol, that is sufficient to supply the increase in borneol (Fig. 5).

Variation in the leaf oil components is shown in Table 2. These results are very similar to the data based on mg/g DW basis (Table 1) and again show that the major effects were between 24 and 48 h of drying. It is interesting that, in the present study and others (Achak et al. 2008, 2009; Adams 2010, 2012, 2013), decomposition products have not been reported to occur during leaf drying. Thus, the characteristic taxon-specific terpene profile has not been lost.

The present study suggests that the changes reported to occur between fresh and 0.5 mo. (Adams, 2013), in fact, occurred when the leaves became dried to point of being brittle (24-48 h). Unfortunately, to preserve specimens for shipping and subsequent herbarium conservation, the leaves must be sufficiently dry so that mold and mildew do not grow.

For chemosystematic studies, the changes found in leaf oil composition during drying do not appear to prohibit their use for taxonomic purposes (Fig. 6). However, for studies of geographic variation (infra-specific), it seems prudent to use either all fresh or all dried materials.

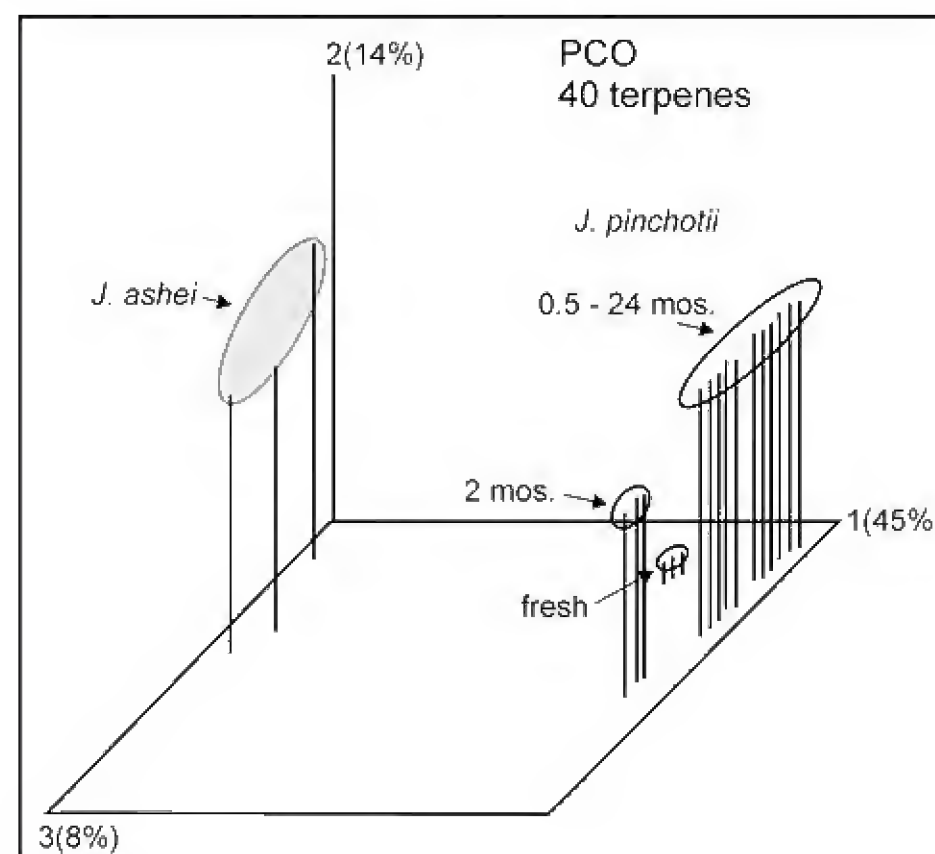


Figure 6. PCO ordination of *J. pinchotii* (oils from fresh and dried leaves) vs. *J. ashei* (fresh leaves). From Adams (2013).

ACKNOWLEDGEMENTS

Thanks to Lawrence Cool and Billie Turner for reviews. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Table 1 Comparison of leaf oils (mg/g x10 basis) for major components obtained from fresh leaves of *J. pinchotii* vs. leaves dried at 42-45° C for 4, 8, 12, 24, and 48 hours. ODW = oven dry wt. of extracted foliage. F sig = F ratio significance, P= 0.05 = *, P= 0.01 = **, ns = non significant, nt = not tested.

KI	Compound	Fresh	4 hr	8 hr	12 hr	24 hr	48 hr	F ratio	F sig
	yield mg/g ODW	16.4	14.5	14.7	16.6	15.7	12.9	7.97	**
924	α -thujene mg/g (x 10)	1.31	1.04	1.07	1.24	1.25	1.42	4.99	**
932	α -pinene	2.23	1.88	1.99	2.07	2.26	2.45	3.41	*
969	sabinene	42.55	40.30	44.10	48.90	46.67	37.19	11.64	**
988	myrcene	4.34	3.91	4.12	4.90	4.54	4.39	6.57	**
1014	α -terpinene	2.29	1.74	1.61	1.91	1.67	1.55	26.87	**
1024	limonene	5.82	5.22	5.51	6.13	5.87	5.22	6.19	**
1054	γ -terpinene	3.59	2.82	2.65	2.42	2.66	2.45	8.78	**
1065	cis-sabinene hydrate	2.54	2.39	2.35	2.68	2.35	1.74	11.98	**
1086	terpinolene	1.63	1.45	1.47	1.54	1.56	1.42	3.61	*
1095	trans-sabinene hydrate	1.06	0.95	0.99	1.21	0.94	0.77	5.15	**
1141	camphor	42.23	37.02	33.21	41.42	36.92	27.25	18.11	**
1145	camphene hydrate	1.23	1.16	1.10	1.42	1.18	0.90	2.90	ns
1148	citronellal	1.23	1.85	2.57	2.06	1.64	1.49	3.86	*
1165	borneol	1.65	1.46	1.69	2.08	2.58	2.52	19.94	**
1166	coahuilensol	1.56	1.17	1.25	1.77	1.49	1.35	3.60	*
1174	terpinen-4-ol	9.15	6.69	5.74	5.12	5.95	4.64	4.66	*
1223	citronellol	5.92	5.89	6.39	6.63	5.87	4.13	12.46	**
1284	bornyl acetate	7.26	7.26	7.85	8.72	8.32	5.74	7.81	**
1548	elemol	8.62	8.75	8.09	9.06	9.40	6.91	4.33	*
1630	γ -eudesmol	0.74	0.73	0.52	0.66	0.63	0.51	3.67	*
1649	β -eudesmol	0.99	0.80	0.81	0.92	1.10	1.04	3.53	*
1652	α -eudesmol	1.07	0.80	0.81	0.89	1.10	0.97	5.41	**
1987	manoyl oxide	1.26	0.78	0.88	0.75	1.02	1.29	1.55	ns

KI = Kovats Index (linear) on DB-5 column.

Table 2. Comparison of leaf oils components (percent total oil basis) obtained from fresh leaves of *J. pinchotii* vs. leaves dried at 42-45° C for 4, 8, 12, 24, and 48 hours. ODW = oven dry wt. of extracted foliage. F sig = F ratio significance, P= 0.05 = *; P= 0.01 = **, ns = non significant, nt = not tested. t = trace, <0.1%.

KI	Compound	Fresh	4 h	8 h	12 h	24 h	48 h	F ratio	F sig
	percent yield (% ODW basis)	1.64	1.45	1.47	1.66	1.57	1.29	7.97	**
921	tricyclene	0.6	0.4	0.4	0.4	0.4	0.5	----	nt
924	α -thujene	0.8	0.7	0.8	0.8	0.8	1.1	19.57	**
932	α -pinene	1.4	1.3	1.4	1.3	1.5	1.9	10.20	**
946	camphene	0.7	0.6	0.5	0.5	0.5	0.5	----	nt
969	sabinene	25.9	27.8	30.0	29.5	29.8	28.9	45.21	**
974	β -pinene	t	t	t	t	t	t	----	nt
988	myrcene	2.7	2.7	2.8	3.0	2.9	3.4	35.04	**
1002	α -phellandrene	t	t	t	t	t	t	----	nt
1014	α -terpinene	1.4	1.2	1.1	1.2	1.1	1.2	9.63	**
1020	p-cymene	t	t	t	t	t	t	----	nt
1024	limonene	3.5	3.6	3.8	3.7	3.8	4.1	10.52	**
1054	γ -terpinene	2.0	2.09	1.8	1.5	1.7	1.9	10.19	**
1065	cis-sabinene hydrate	1.5	1.7	1.6	1.6	1.5	1.4	9.13	**
1086	terpinolene	1.0	1.0	1.0	1.0	1.0	1.1	1.93	ns
1098	trans-sabinene hydrate	0.7	0.7	0.7	0.7	0.6	0.6	2.53	ns
1118	cis-p-menth-2-en-1-ol	0.4	0.4	0.3	0.4	0.3	0.4	----	nt
1141	camphor	25.7	25.6	22.6	24.8	23.6	21.1	10.17	**
1145	camphene hydrate	0.8	0.8	0.8	0.9	0.8	0.7	1.37	ns
1148	citronellal	0.8	1.3	1.8	1.3	1.1	1.2	4.43	*
1165	borneol	1.0	1.0	1.2	1.3	1.7	2.0	89.21	**
1166	coahuilensol	0.9	0.8	0.9	1.1	1.0	1.1	3.59	*
1174	terpinen-4-ol	5.6	4.6	3.9	3.1	3.8	3.6	3.96	*
1186	α -terpineol	0.3	0.3	0.2	0.3	0.3	0.3	----	nt
1195	cis-piperitol	t	t	t	t	t	t	----	nt
1207	trans-piperitol	t	t	t	t	t	t	----	nt
1219	coahuilensol, me-ether	t	t	t	t	t	t	----	nt
1223	citronellol	3.6	4.0	4.4	4.0	3.8	3.2	6.14	**
1274	pregeijerene B	t	t	t	t	t	t	----	nt
1284	bornyl acetate	5.0	5.0	5.4	5.3	5.3	4.5	10.14	**
1298	carvacrol	t	t	t	t	t	t	----	nt
1374	α -copaene	t	t	t	t	t	t	----	nt
1451	trans-muurolo-3,5-diene	t	t	t	t	t	t	----	nt
1475	trans-cadina-1(6),4-diene	t	t	t	t	t	t	----	nt
1493	trans-muurolo-4,5-diene	t	t	t	t	t	t	----	nt
1493	epi-cubebol	t	t	t	t	t	t	----	nt
1500	α -muurolene	t	t	t	t	t	t	----	nt
1514	cubebol	t	t	t	t	t	t	----	nt
1522	δ -cadinene	t	t	t	t	t	t	----	nt
1548	elemol	5.3	6.0	5.5	5.5	6.0	5.4	1.46	ns
1559	germacrene B	0.3	t	t	t	t	0.4		
1627	1-epi-cubenol	t	t	0.3	t	t	0.3		
1630	γ -eudesmol	0.5	0.5	0.4	0.4	0.4	0.4	3.20	*
1649	β -eudesmol	0.6	0.6	0.6	0.6	0.7	0.8	8.60	**
1652	α -eudesmol	0.7	0.6	0.6	0.5	0.7	0.8	9.43	**
1670	bulnesol	t	t	t	t	t	t	----	nt
1987	manoyl oxide	0.8	0.5	0.6	0.5	0.7	1.0	3.72	*
2055	abietatriene	t	t	t	t	t	t	----	nt
2087	abietadiene	0.2	t	t	t	t	0.1	----	nt
2298	4-epi-abietal	0.4	0.1	0.2	0.1	0.2	0.3	----	nt
2312	abieta-7,13-dien-3-one + abietal	0.5	0.3	0.2	0.2	0.3	0.3	----	nt

A new species of *Lepechinia* (Lamiaceae) from Oaxaca, Mexico

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ABSTRACT

A new taxon, ***Lepechinia oaxacana*** B.L. Turner, **sp. nov.**, is described from southern Oaxaca. It is closely related to the recently described *L. flammea* of Guerrero, but differs in a syndrome of characters, mainly leaf size and floral features. A photograph of the holotype is provided, along with a distribution map of the taxa concerned. Published on-line: www.phytologia.org *Phytologia* 95(2): 138-140 (May 1, 2013).

KEY WORDS: Lamiaceae, Mexico, Guerrero, Oaxaca, *Lepechinia*, *L. flammea*, *L. glomerata*, *L. oaxacana*

Preoccupation with the Mexican species, *Lepechinia glomerata* Epling and cohorts, these clearly related to the Californian *L. calycina*, as documented by the DNA studies of Drew and Sytsma (2011), has occasioned the present paper. Early on, I was prepared to describe a novel taxon from the state of Guerrero, noting its many differences from the poorly known *L. glomerata*, but was pre-empted by the excellent study of Martinez-Gordillo and Lozado-Perez (2009), who proposed the name *L. flammea*, for my intended novelty (this to be based upon a single sheet, *Martinez et al.* 4933, TEX). Recent collections from Oaxaca, gathered through the support of SERBO, have revealed an additional novelty of the *L. glomerata* complex, this described below.

LEPECHINIA OAXACANA B.L. Turner, **sp. nov.** Fig. 1

Perennial herbs, to “1.5 m” high. **Stems** (upper), sparsely pubescent with crinkly, often branched, trichomes, 1.0-1.4 mm high, beneath these a denser array of minute glandular hairs ca 0.3 mm high. **Leaves** sessile, amplexicaule, 6-10 cm long, 2-3 cm wide; blades elliptic-lanceolate, widest near the middle, pubescent above and below, mainly along the venation. **Inflorescence** terminal, 10-15 cm high, 5-7 cm wide; peduncles ca 3 cm long; floral bracts (outer) broadly ovate, 8-10 mm long, and as wide, dark purplish; floriferous branches 2-3 cm long, each bearing 10-15 flowers, the ultimate pedicels ca 1 mm long. **Calyces** (flowering), ca 6 mm long; lobes 5, united for ca 3 mm, their apices triangular to lanceolate. **Corollas** 10-12 mm long; tube ca 3 mm long; throats “anaranjada,” 8-9 mm long, 3-4 mm wide, their apices not rosy in color (as in *L. flammea*). **Anthers** purple, ca 1 mm long, excurrent for 3-5 mm. **Nutlets**, black, smooth, 2.1-2.6 mm long, 1.5-2.0 mm wide.

TYPE: MEXICO. OAXACA: Distrito Sola de Vega, Mpio. Santiago Textitlan, “Paraje abajo de El Portillo, Bosque de pino-encino. Suelo negro.” ca 1190 m, 16 43 58.2 N, 27 25 10.3 W, 08/01/07, *Idalia Trujillo Olazo* (ITO) 1336 (Holotype: TEX).

Lepechinia oaxacana is clearly closely related to the recently described *L. flammea* of Guerrero (Martinez-Gordillo and Lozado-Perez, 2009). The novelty differs in having a syndrome of distinctive characters: smaller foliage (mostly 6-10 cm long, 2-3 cm wide vs 10-20 cm long, 3-6 cm wide) smaller calyces (ca 6 mm long vs 9-11 mm); smaller corollas (10-12 mm long, 3-4 mm wide vs 16-20 mm long, 6-8 mm wide), their apices not notably rose-colored, or flame-like, as in *L. flammea* (hence its name).

The novelty is said to occur in pine-oak woodlands at an elevation of ca 1190 m; *L. flammea* reportedly occurs at somewhat higher elevations (2000-2700 m).

ACKNOWLEDGEMENTS

I am grateful to my field companion, Jana Kos, for helpful editorial suggestions, and to the following herbaria for the loan of specimens: ARIZ, ASU, CAS and UC.

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Fig. 1. *Lepechinia oaxacana* (Holotype: TEX).



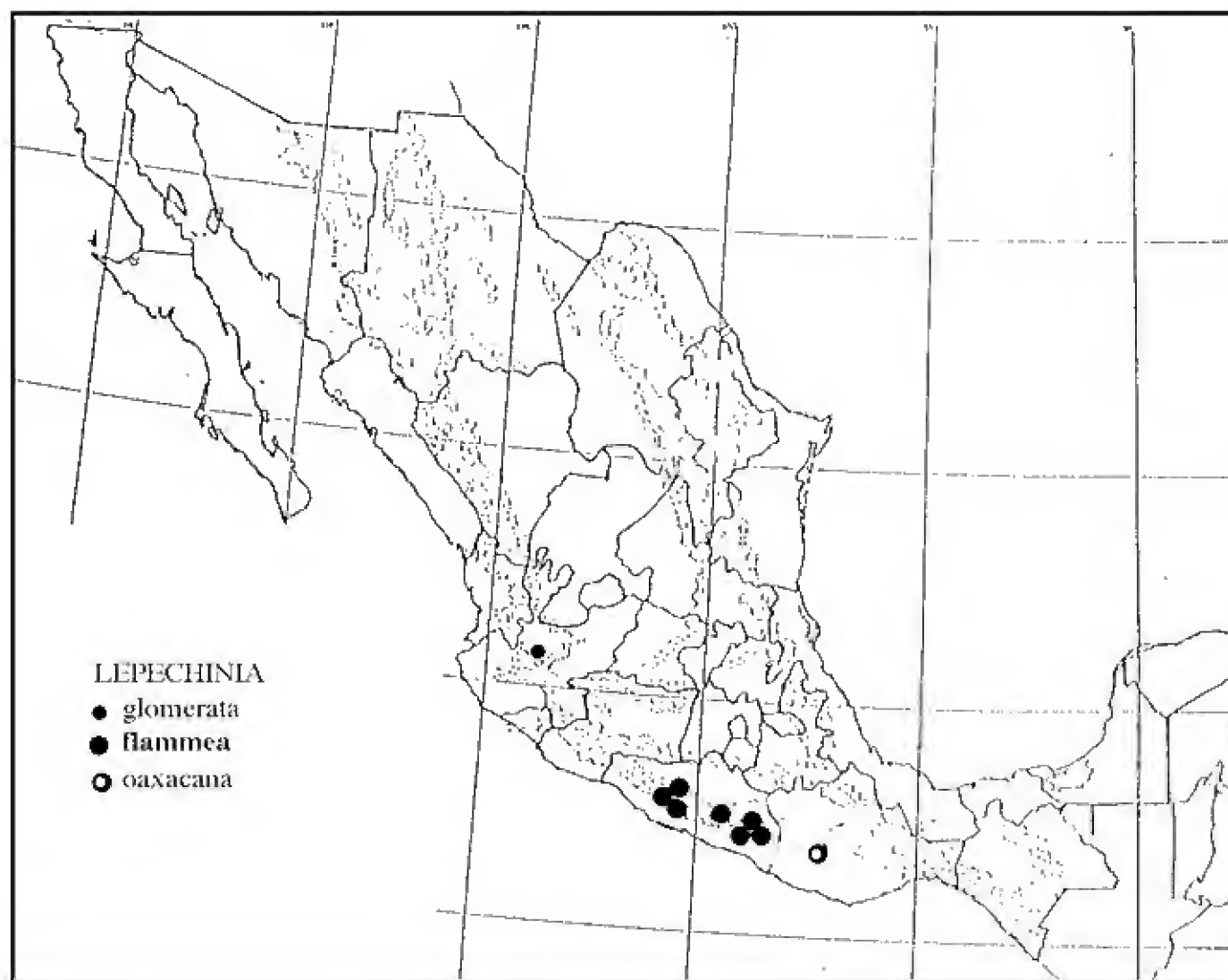


Fig. 2. Distribution of the *Lepechinia glomerata* complex in Mexico.

Five new species of *Ageratina* (Asteraceae: Eupatorieae) from Oaxaca, Mexico

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ABSTRACT

Five new taxa of *Ageratina* are described from Oaxaca, Mexico: *Ageratina cuicatlan* B.L. Turner, sp. nov.; *Ageratina etlana* B.L. Turner, sp. nov.; *Ageratina megaphylla* B.L. Turner, sp. nov.; *Ageratina pauciflora* B.L. Turner, sp. nov.; *Ageratina tejalpana* B.L. Turner, sp. nov. Photographs of the types are presented, along with appropriate maps showing their distribution (vis a vis closely related taxa). www.phytologia.org *Phytologia* 95(2): 141 - 150 (May 1, 2013).

KEY WORDS: Asteraceae, Eupatorieae, Mexico, Oaxaca, *Ageratina*.

Preoccupation with the study of a potpourri of Mexican Asteraceae from the state of Oaxaca, sent to the author by SERBO for identification, has occasioned the present paper.

AGERATINA CUICATLANA B.L. Turner sp. nov. Fig. 1

Stiffly erect perennial herbs to 40 cm high. **Stems** pubescent with minute coarse hairs, the vestiture ca 0.2 mm high. **Leaves** (upper) mostly alternate, 2-4 cm long, 1-2 cm wide; petioles 0.5-1.0 cm long; blades ovate, both surfaces somewhat glutinous and glandular-punctate, glabrous or nearly so, 3-nervate from the very base, the margins minutely serrate. **Capitulescence** a terminal cymose panicle ca 8 cm high, 3-8 cm wide, the ultimate peduncles 5-15 mm long. **Heads** cylindric, ca 7-8 mm high, 3-5 mm wide; involucre bracts ca 10, 2-seriate, linear-lanceolate, 3-nervate. **Receptacle**, plane, viscid, ca 1 mm across. **Florets** 8-10 per head; corollas white, glabrous, 4-5 mm long; tubes ca 2 mm long, the 5 lobes ca 1 mm long. **Achenes** viscid, apically pubescent, ca 2 mm long; pappus of ca 40 stiff, persistent, somewhat tawny bristles ca 5 mm long.

TYPE: MEXICO. OAXACA: Distrito Cuicatlan, Mpio. San Juan Bautista Cuicatlan, “12.9 km de la Cieneguilla, bajada a Santa Catarina Tlaxila.” ca 2095 m, 17 29 43.5 N, 97 00 1.1 W, “Selva baja caducifolia secundario.” 8 Nov 2001, *Silvia H, Salas M.* 4353 [with Schibli & Chemnick] (Holotype: MEXU; isotype: TEX). **Map 1**

This species belongs to the subgenus *Neogreenella*, nesting among the alternate leafed complex, somewhere near *A. hyssopifolia* (Gray) King & Rob. and *A. thrysiflora* (Greene) King & Rob., to which it will key in my treatment of *Ageratina* for Mexico (Turner 1997); the latter two taxa are confined to northwestern Mexico, and bear little resemblance to the present novelty.

The name refers to the Distrito Cuicatlan, whence the type.

AGERATINA ETLANA B.L. Turner, sp. nov. Fig. 2

Perennial herbs to 1 m high. **Stems**, densely glandular-pubescent, the vestiture ca 0.3 mm high. **Leaves** (upper) 6-7 cm long, 2-3 cm wide; petioles 1.5-3.0 cm long; blades ovate-lanceolate, 3-nervate from the base, upper surfaces weakly pubescent, lower surfaces glandular pubescent, mainly along the veins, the margins serrulate. **Capitulescence** a terminal cymose panicle of 10-20 heads, 3-4 cm high, 4-5 cm across, the ultimate peduncles 5-10 mm long, beset with 3-6 linear bracts. **Heads**, 4-5 mm high, 5-6 mm wide; involucre bracts 2-seriate, 3-4 mm long, linear-lanceolate, markedly glandular-pubescent. **Florets** ca 40 per head; corollas glabrous, ca 4 mm long, pinkish white (dried); tubes much-narrowed, 1.5- 2.0

mm long; throat campanulate, ca 1.5 mm long; lobes 5, ca 0.5 mm long, glabrous, or nearly so. **Anthers** yellow, the appendages ovate. **Achenes**, black, glabrous, ca 1.5 mm long; pappus of ca 20 readily deciduous bristles ca 4 mm long.

TYPE: MEXICO. OAXACA: Distrito Etla, Mpio. San Felipe Tejalapa, “El Timbre,” ca 1819 m, 17 03 06.4 N, 96 53 39.4 W, 14 Feb 2012, Mario Cruz Cruz [MAC] 878 (holotype: TEX). **Map 2**

According to the collector, the plant occurred in a “Bosque de pino-encino. orilla de rio.”

In my treatment of *Ageratina* for Mexico, largely because of its densely glandular-pubescent stems and foliage, the novelty will key to *A. zunilana* (Standl. & Steyerl.) King & Rob., a species of Chiapas and Guatemala having much larger heads (7-8 mm high vs 4-5 mm), pubescent achenes (vs glabrous), among yet other characters.

The name refers to the Distrito Etla, whence the Type.

AGERATINA MEGAPHYLLA B.L. Turner, sp. nov. Fig. 3

Shrubs 2 m high. **Stems** (upper), ca 5 mm thick, densely pubescent with tawny, spreading hairs, the vestiture 1-2 mm high. **Leaves** (mid-stem), opposite, ca 17 cm long, 9 cm wide; petioles 2.5-3.0 cm long, pubescent like the stems; blades broadly ovate, pinnately nervate, sparsely pubescent above, more densely so below, especially along the venation; margins irregularly serrate. **Capitulescences** axillary, cymose-paniculate, the ultimate peduncles mostly 2-5 mm long. **Heads**, 4-5 mm high; involucre bracts lanceolate, 4-5 mm long, ca 0.5 mm wide, apically acute, sparsely pubescent. **Florets**, ca 20 per head; corollas, ca 3 mm long, glabrous, except for the pubescent lobes. **Achenes** (immature) ca 2 mm long, sparsely pubescent; pappus of ca 30, very fragile, ciliate, white bristles ca 3 mm long.

TYPE: MEXICO. OAXACA: Distrito Etla; Mpio. San Felipe Tejalapa. Captacion de agua El Negro. “Bosque de pino-encino. Orilla de arroyo, en cascajo negro.” 17.1 25.7 N, 96.55 0.7 W, ca 2210 m, 17/02/2012, Mario Cruz Cruz [MC] 993 (Holotype: TEX). **Map 1**

In my treatment of *Ageratina* (subgenus *Ageratina*) for Mexico (Turner 1997), the present novelty will key to or near *A. peracuminata* King & Rob., an herbaceous species of south-central Oaxaca having much thinner, smaller, somewhat deltoid, sparingly pubescent, leaf blades. *Ageratina megaphylla* is described by its collector, as a shrub “2 m” high; it is perhaps best recognized by its large leaves (hence the appellation), and densely pubescent stems and petioles.

AGERATINA PAUCIFLORA B.L. Turner, sp. nov. Fig. 4

“**Arbol**” to 3 m high. **Leaves** (upper) opposite, pinnatinervate, 12-18 cm long, 5-7 cm wide; petioles 2-3 cm long, narrowly winged and grading into the blades, glabrous and glandular-punctate above and below, their margins irregularly serrate. **Capitulescence**, a terminal, cymose-panicle ca 5 cm high, 7 cm wide, the ultimate peduncles minutely glandular pubescent, viscid, 2-5 mm long. **Heads** narrowly campanulate, 6-7 mm high, ca 3 mm wide; involucre ca 4 mm long, composed of 5-7, linear-lanceolate, bracts arranged in 2 series, their apices acute to obtuse. **Florets** 2-4(5) per head, glabrous; corollas white, glabrous, ca 3 mm long; throat ca 0.75 mm long, the lobes 5, ca 1 mm long. **Stamens** scarcely exerted, if at all, anthers yellow, their apices ovate, ca as wide as long. **Achenes** ca 2 mm long, glabrous; pappus of ca 20 persistent bristles, ca 4 mm long.

TYPE: MEXICO. OAXACA: Distrito, Sola de Vega, Mpio. Santiago Textitlan. "Paraje La Fragua." ca 2335 m, 16 47 12.1 N, 97 18 57.4 W, 14 Mar 2007, *Arturo Sanchez Martinez 2079* [with Ana Ruiz & Mayra Hernandez] (Holotype: TEX)

The species reportedly occurs in pine-oak forests. It is named for its relatively few-flowered heads.

Ageratina pauciflora clearly belongs to the *A. ligustrina* (DC.) King & Rob. complex, a wide-ranging, highly variable assemblage, as envisioned by Turner (1997). It can be readily separated from the former by its very large leaves, smaller, fewer-headed capitulescences, and smaller heads with fewer florets. The distribution of the two taxa in Oaxaca also differs, as indicated in **Map 4**.

AGERATINA TEJALAPANA B.L. Turner, sp. nov. Fig. 5

Perennial herbs to "1 m" high. **Mid-stems** minutely pubescent. **Leaves** opposite, 4-5 cm long, 3-4 cm wide; petioles 5-12 mm long, pubescent like the stems; blades subcordate, glabrous above and below, or nearly so; margins serrate, the teeth obtuse to rounded. **Capitulescence** a terminal, cymose panicle, ca 25 cm high and as wide, the ultimate peduncles 1-4 cm long. **Heads** campanulate, 5-6 mm high, ca 8 mm across; involucre bracts biseriate, the bracts linear-lanceolate, glabrous, 4-5 mm long, 1.0-1.5 mm wide, the apices acute to obtuse. **Florets** white, 50-60 per head; corollas ca 3.5 mm long, glabrous, except for the pubescent lobes. **Achenes** (immature) sparsely pubescent, ca 1.5 mm long; pappus of ca 20 readily deciduous white bristles ca 3 mm long.

TYPE: MEXICO. OAXACA: Distrito Etlá, Mpio. San Felipe Tejalapa, "Loma de la Mina. Bosque de Encino-pino, sobre cerro, suelo amarillo." 17 04 9.4 N, 96 53 23.9 W, ca 1833 m, 17 Oct 2011, *Cleotilde Cervantes Morales* [CLEO] 558 (Holotype: TEX).

This novelty will key to *A. choriccephala* in my treatment of Mexican *Ageratina* (Turner 1997), a more northern species having a smaller capitulescence with smaller, fewer-flowered heads and fewer involucre bracts. Distribution of the two taxa is shown in **Map 4**.

The species name derives from the Mpio. San Felipe Tejalapa, whence the Type.

As noted by Turner (2010), "*Ageratina* is perhaps the most speciose genus of the Asteraceae in Mexico;" with description of the five taxa herein, the generic number currently stands at ca 190.

ACKNOWLEDGEMENTS

Thanks to SERBO for the collections concerned, and to my close consort, Jana Kos, for editing the paper. The distribution map is based upon collections at LL-TEX).

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Fig. 1 *AGERATINA CUICATLANA* B.L. Turner sp. nov.



Fig. 2 AGERATINA ETLANA B.L. Turner, sp. nov.

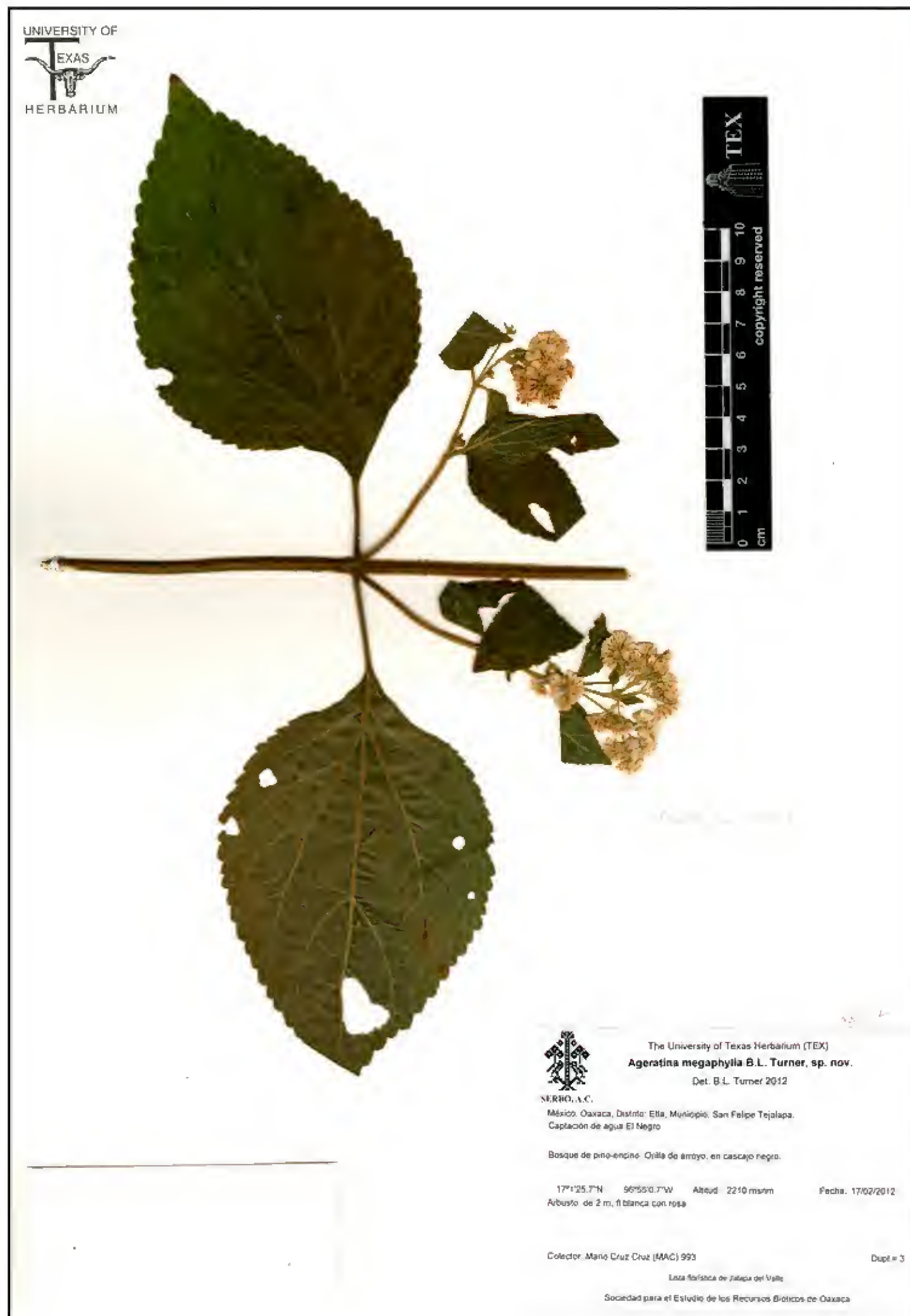


Fig. 3 *AGERATINA MEGAPHYLLA* B.L. Turner, **sp. nov.**



Fig. 4 AGERATINA PAUCIFLORA B.L. Turner, sp. nov.

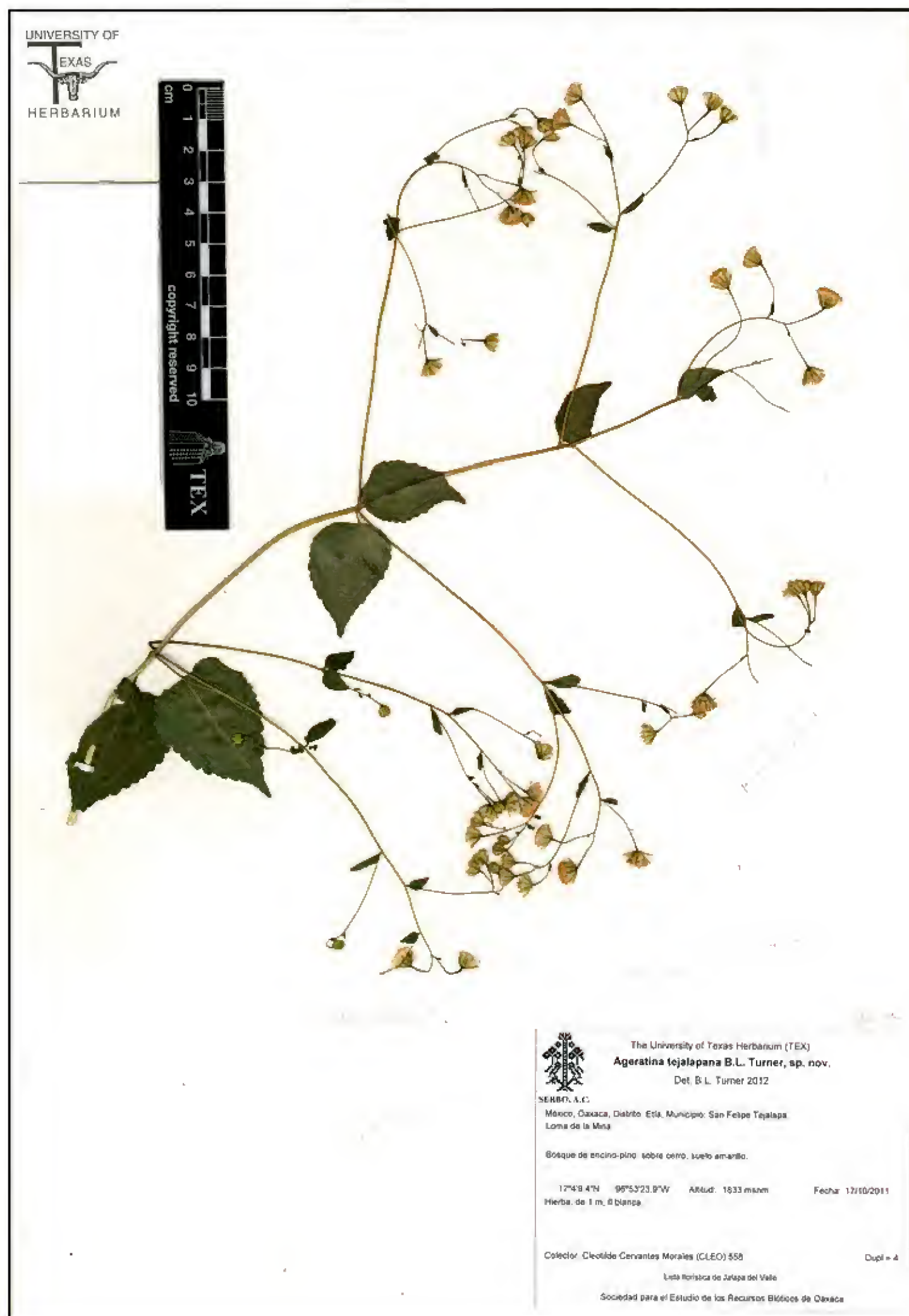
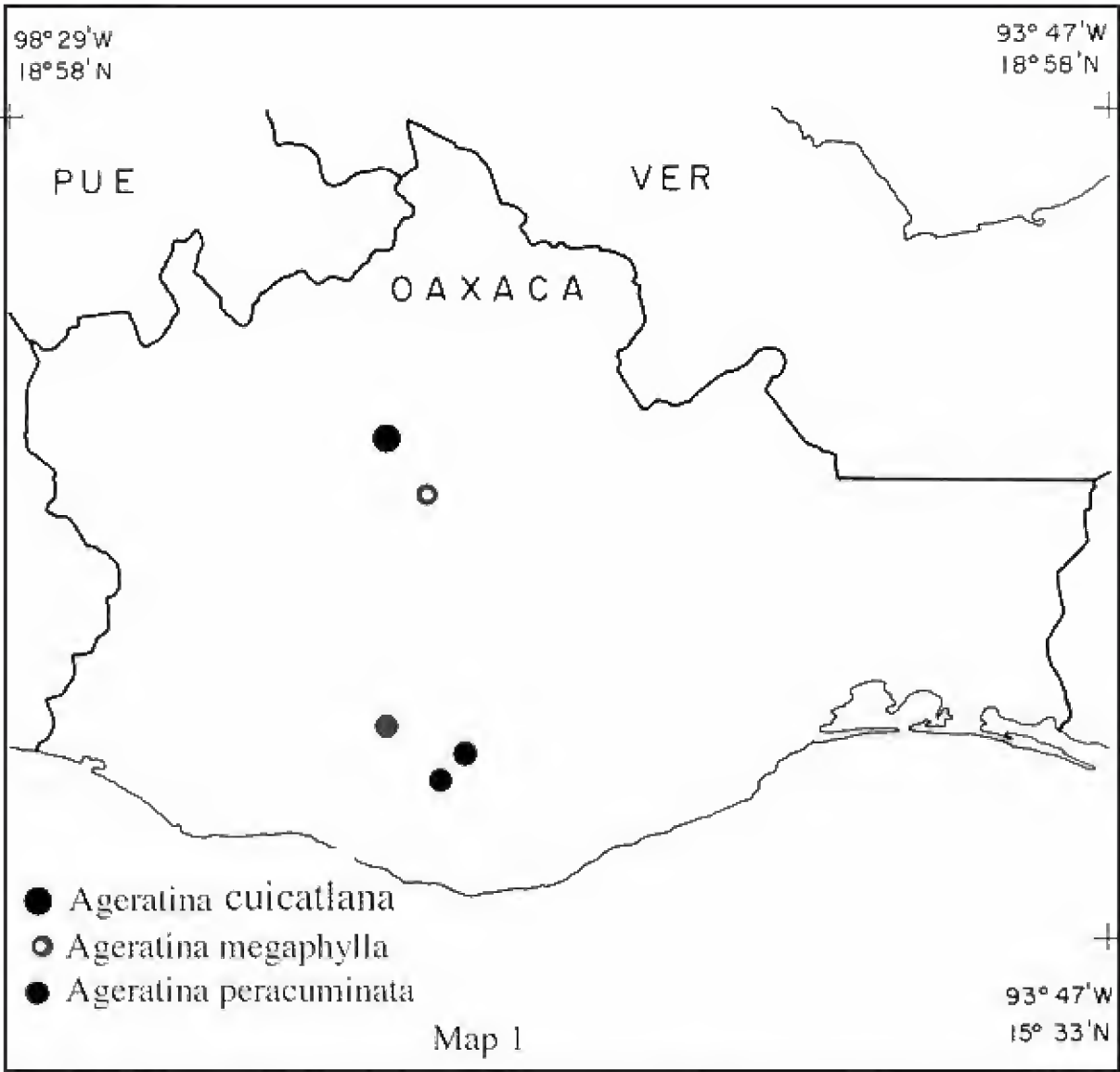
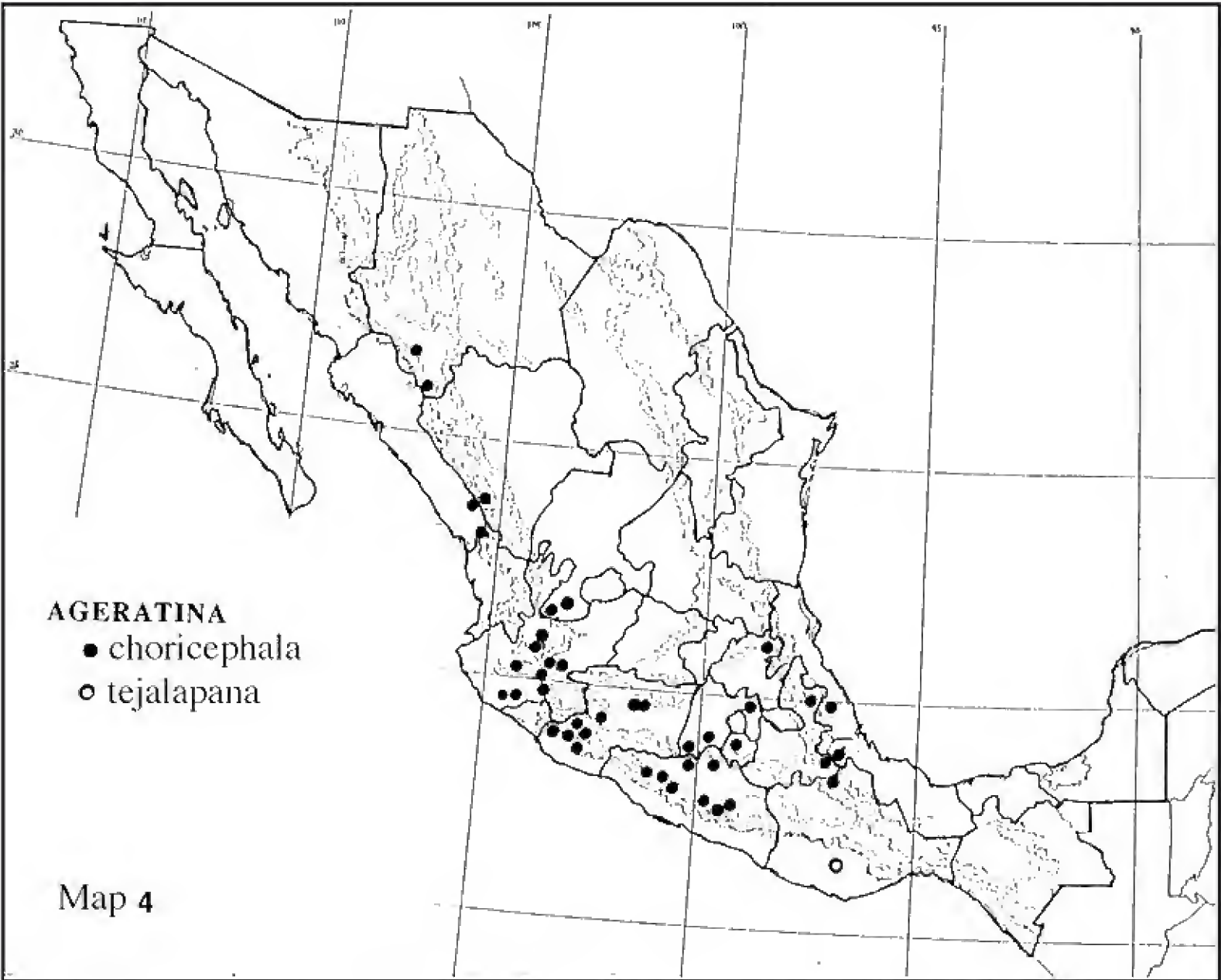
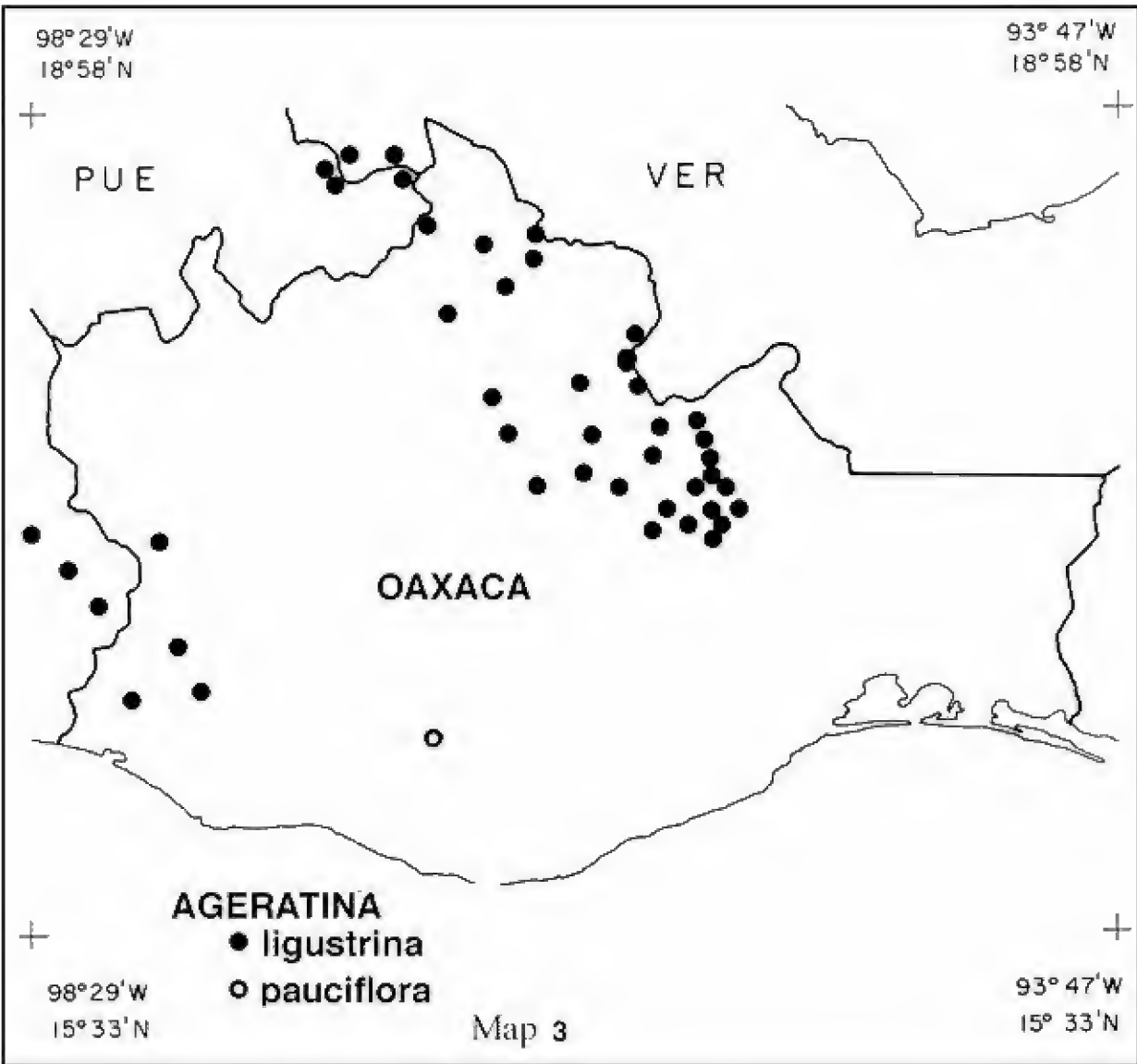


Fig. 5 *AGERATINA TEJALAPANA* B.L. Turner, sp. nov.





Recension of Mexican species of *Otopappus* (Asteraceae, Heliantheae)

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ABSTRACT

A taxonomic treatment of the Mexican species of *Otopappus* is rendered. With the positioning of two species into yet other genera as advocated by Strother (1999) and Panero (2007), and the description of a new taxon, ***Otopappus serboana*** B.L. Turner, **sp. nov.** from the state of Oaxaca, Mexican species now number 14. A photograph of the novelty is provided, along with distribution maps of the taxa concerned. The treatment is presented in the format of the author's on-going Comps of Mexico. www.phytologia.org *Phytologia* 95(2): 151-160 (May 1, 2013).

KEY WORDS: Asteraceae, Heliantheae, *Otopappus*, Mexico, Oaxaca

OTOPAPPUS Benth.

Notoptera Urban

Shrubs, or scrambling or clambering tree-like leaners to 10 m high. Leaves opposite, simple, 3-nervate to subpinnately reticulate-veined with mostly harsh hairs (rarely not). Heads small to large, radiate or rarely not, 1-numerous in terminal or subterminal cymules. Involucres campanulate to hemispheric, 4-6 seriate, mostly strongly graduate, but the outermost series sometimes loose and leafy, longer than the head itself. Receptacles convex, paleate. Ray florets yellow, mostly 8-34 (rarely absent or much-reduced) pistillate, fertile. Disk florets white or yellow. Achenes, those of the disk, radially flattened to somewhat 3-sided with winged margins, these extending onto the 1 or 2 lateral awns, between the latter occur several or more, short scales, these often united into a crown. Base chromosome number, $x = 16$.

Type species, *Otopappus verbosinoides* Benth.

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 McVaugh, R. 1984. *Otopappus*, in Flora Novo-Galiciana 12: 648-658.
 Panero, J.L. 2007. *Tuxtla*, in Anderberg et al., Families and Genera Vascular Plants 8: 458.
 Strother, J.L. 1999. *Otopappus*, in Flora Chiapas 5: 82-84.
 Villasenor, J.L. and J.L. Strother. 1989. *Tuxtla*, a new genus for *Zexmenia pittieri* (Compositae: Heliantheae). Syst. Bot. 14: 529-540.

A genus of mostly shrubs or scrambling vine-like, clambering, plants to 10 m high. Hartman and Stuessy (1983) recognized 15 species for the genus, 13 of which occurred in Mexico. Subsequently, two of the Mexican species were transferred to other genera. Thus, McVaugh's *Otopappus jaliscensis*, was treated as belonging to the genus *Lasianthaea* by Hartman and Stuessy (1983); Strother (1999), however, positioned the taxon in the genus ***Lundellianthus***, where its true position seems to be. Likewise, *Otopappus pittieri* (Greenm.) B.L. Turner has recently been transferred to the monotypic genus ***Tuxtla*** (Strother 1999). As of now, with the description of ***O. serboana*** (below), the number of Mexican species appears to be 14.

KEY TO SPECIES

1. Heads radiate, if rays absent then the disk corollas yellow ...(4)
1. Heads eradiate; corollas white ...(2)
 2. Disk corollas not recurved at maturity;
involucral bracts of middle and outer series 1/2
or less as long as the inner bracts and pales; Cam,
Yuc, Qui**O. guatemalensis**
 2. Disk corollas markedly recurved at maturity;
involucral bracts grading into the pales, not
markedly set off as to size or texture; Ver, Oax, Tab, Cps ...(3)
3. Heads on ultimate peduncles mostly 5-15 mm long.....**O. curviflorus**
3. Heads on ultimate peduncles 0-5 mm long**O. brevipes**
- 4(1). Ray florets present, usually well-developed, pistillate, and fertile...(6)
4. Ray florets absent or much reduced and neuter, sterile ...(5)
5. Heads mostly in axillary clusters of 2-5;
receptacular pales with subulate, markedly recurved,
apices; Gue, Oax**O. mexicanus**
5. Heads 5-15 in both terminal and axillary clusters;
receptacular pales with ovate, mostly erect, apices,
or nearly so**O. robustus**
- 6(4). Involucres 5-10 mm high, 7-15 mm wide ...(7b)
6. Involucres 3-5(7) mm high, 4-7 mm wide...(7a)
- 7a. Ray florets 30 +; disc florets 80 +; pales 6-7 mm long; Oax**O. serboanus**
- 7a. Ray florets 13-15; disc florets 40-75; pales ca 5 mm long.....**O. microcephalus**
- 7b. Receptacular pales erect, bristly-terete at the apices;
leaves softly and densely pilose beneath; Jal **O. acuminatus**
- 7b. Receptacular pales not as above, usually abruptly
narrowed or flattened at the apices; leaves mostly
sparsely to moderately pubescent beneath and rough to the touch ...(8)
8. Outermost involucral bracts appressed, or if
somewhat loose then ovate to linear to linear-
oblanceolate, not usually as long as the inner bracts...(10)
8. Outermost involucral bracts, oblanceolate to
spatulate, loose, and foliaceous, often longer than
the innermost involucral bracts ...(9)
9. Leaves smooth to slightly scabrous above with
closely appressed hairs, the latter without enlarged
basal cells; ray florets 8-14**O. verbesinoides**

9. Leaves markedly scabrous above with erect or ascending hairs, the latter with enlarged basal cells; ray florets mostly 15-28**O. scaber**
- 10(8). Margins of the leaf coarsely and irregularly dentate, the blades felty-pubescent beneath, mostly 1.5-2.0 times as long as wide; Gue, Mex, Mor, Pue**O. imbricatus**
10. Margins finely serrulate to nearly entire, the blades coarsely-pubescent beneath to nearly glabrous, mostly 2-4 times as long as wide ...(11)
11. Involucres mostly 12-20 mm wide ...(13)
11. Involucres mostly 6-10 mm wide ...(12)
12. Leaves elliptical, broadest at or near the middle; Ver[*O. pittieri*] **Tuxtla pittieri**
12. Leaves ovate, broadest at or near the base; Sin to Gue**O. tequilanus**
- 13(11). Leaves strigose on both surfaces with closely appressed hairs; ligules 15-18 mm long**O. koelzii**
13. Leaves scabrous, the lower surface with erect or ascending hairs; ligules 5-9 mm long**O. epaleaceus**

OTOPAPPUS ACUMINATUS S. Wats., Proc. Amer. Acad. Arts 26: 140. 1891.

Notoptera tequilana var. *acuminata* (S. Wats.) Blake

Otopappus tequilanus var. *acuminatus* (S. Wats.) B.L. Rob.

Known only from Jal, subtropical deciduous forests, steep slopes, 1200-1700 m; Jul-Oct. **Map 1**

Much resembling **O. tequilanus** but the receptacular bracts stiffly terete at the apices and the leaves densely softly pilose beneath; chromosome number, n = 16 pairs.

Hartman and Stuessy (1983) point out the distinctions between this taxon and **O. tequilanus**. McVaugh (1984) notes that the latter, quite variable, species occurs mostly at lower elevations (100-1300 m) along the Pacific slopes while the more uniform **O. acuminatus** appears to occur at higher elevations.

OTOPAPPUS BREVIPES B.L. Rob., Proc. Amer. Acad. Arts 44: 621. 1909.

Notoptera brevipes (B.L. Rob.) Blake

Otopappus glabratus (J. Coulter) Blake

Otopappus brevipes var. *glabratus* (J. Coulter) B.L. Rob.

Cps and Guatemala southwards, montane rain forests, 800-2100 m; Nov-Jan. **Map 1**

Much resembling **O. curviflorus** but the heads smaller, nearly sessile, and the corollas with broader throats and shorter lobes.

According to Hartman and Stuessy (1983), **O. brevipes** occurs at, generally, higher altitudes than **O. curviflorus** (450-2100 m vs 20-1300 m).

OTOPAPPUS CURVIFLORUS (R. Br.) Hemsl., Biol. Centr. Amer. Bot. 2: 191. 1881.

Notoptera curviflora (R. Br.) Blake

Notoptera scabridula Blake

Ver, Oax, Tab, Cps, Cam, Qui and Guatemala southwards, tropical lowland forests, 20-1000 m; Nov-Jun. **Map 1**

Erect or clambering shrubs 4-8 m high; leaves 4-18 cm long, 1-6 cm wide; petioles 3-18 mm long; blades ovate to broadly lanceolate, pinnately veined, mostly softly villous beneath, the margins serrate to nearly entire; heads campanulate, rayless, arranged in pyramidal corymbose panicles, the ultimate peduncles mostly 5-15 mm long; involucre 3-4 seriate, the bracts graduate and grading into the pales; ray florets absent; disk florets 30-50, the corollas white and strongly out-curved at maturity; achenes 2-3 mm long, the pappus of 2, unequally winged, awns 0.5-2.0 mm long.

Closely related to *O. brevipes* but clearly distinct and easily recognized by the characters given in the key. According to Strother (1999), however, the "Types of the names *Otopappus brevipes* and *O. curviflorus* may prove to be conspecific."

OTOPAPPUS EPALEACEUS Hemsl., Biol. Centr. Amer. Bot. 2: 191. 1881.

Notoptera epaleaceus (Hemsl.) Blake

Jal, Mic, Mex, Mor, Pue, Gue and Oax, tropical deciduous forests, 25-2000 m; Sep-Dec.

Shrubs, or scrambling vines; leaves 7-15 cm long, 2.5-6.5 cm wide; petioles 5-18 mm long; blades ovate, mostly thick and reticulate beneath, the vestiture rough to the touch, the margins serrulate; heads radiate, 3-7 in terminal or subterminal cymose clusters; involucre broadly campanulate to hemispheric, 8-15 mm high, 12-22 mm wide, the bracts in 6-8 series, graduate, the outermost somewhat loose and mostly 4-8 mm long; ray florets 21-43, the ligules yellow, 3-12 mm long; disk florets numerous (80-120), the corollas yellow; achenes 3-4 mm long, the pappus of 2, winged awns 1.5-3.5 mm long; chromosome number, $n = 16$ pairs.

A variable species but recognized by its few-headed cymules and large, mostly hemispheric, heads with 5-6 seriate involucre, the outer series loose but short. The undersurfaces of the leaves are mostly roughly hispid, but a few recent collections (*Guerrero 1083*, TEX) have a softly pilose vestiture, reminiscent of *O. acuminatus*, but other features are clearly those of *O. epaleaceus*.

OTOPAPPUS GUATEMALENSIS (Urban) Hartman & Stuessy, Syst. Bot. 8: 206. 1983. **Map 2**

Notoptera guatemalensis Urban

Notoptera leptcephala Blake

Cam, Yuc, Qui? adjacent Belize and Guatemala, tropical deciduous forests, 0-300 m; all seasons.

Superficially resembling *O. curviflorus* but readily distinguished by its narrow, few-flowered heads (12-18 florets vs 30-50) and markedly different involucral bracts, as noted in the key to species.

OTOPAPPUS IMBRICATUS (Sch.-Bip.) Blake, Contr. U.S. Natl. Herb. 26: 255. 1930.

Otopappus cordatus Blake

Otopappus epaleaceus var. *pringlei* Greenm.

Otopappus xanthocarphus Brandege

Mic, Mex, Mor, Pue and Gue in tropical deciduous or pine-oak forests, 800-1700 m; Jun-Oct. **Map 2**

Much resembling *O. epaleaceus* but the leaves characteristically strongly and closely dentate, the blades broadly ovate, 1-2 times as long as wide (vs 2-4), the undersurfaces prominently reticulate and mostly felty-pubescent; chromosome number, $n = 16$ pairs.

Vegetatively this appears to be a distinct taxon, the leaves being relatively broad and with strongly dentate margins. Hartman and Stuessy (1983) cite two specimens from Pue, both of which I would place elsewhere (*Torke et al. 319*, in *O. tequilanus*; *Johnston s.n.* in *O. epaleaceus*).

OTOPAPPUS KOELZII McVaugh, Contr. Univ. Michigan Herb. 9: 425. 1972.

Col, Jal and Mic, tropical deciduous forests, Pacific slopes, 50-600 m; Oct-Dec. **Map 2**

Much resembling **O. epaleaceus** but distinguished by its leaves that have relatively smooth undersurfaces with closely appressed, strigose, hairs (as opposed to erect or ascending hairs, especially along the veins), ligules 15-20 mm long (vs 5-10 mm), and disk corollas 6-7 mm long (vs 4-5 mm).

OTOPAPPUS MEXICANUS (Rzed.) H. Rob., Wrightia 6: 44. 1979.

Oyedaea mexicana Rzed.

Gue and Oax, Pacific slopes, tropical deciduous forests, 800-1000 m; Aug-Nov. **Map 2**

Clambering shrubs 1-10 m high; leaves 9-13 cm long, 3.5-5.5 cm wide; petioles 5-10 mm long; blades ovate, 3-nervate from or near the base, sparsely strigillose, the margins serrulate to nearly entire. Heads radiate, 1-5 in the leaf axils, the ultimate peduncles 3-12 mm long; involucre campanulate, 3-5 m high, 3-4 seriate, the bracts graduate; ray florets 8-15, neuter, sterile, the ligules 1-4 mm long, yellow; disk florets 50-80, the corollas yellow, those of the periphery recurved; achenes 2.5-3.0 mm long, the pappus of 2 winged awns, 1.2-2.0 mm long, between these a united crown of scales 0.3-0.8 mm long.

Hartman and Stuessy (1983) cited specimens of this taxon from Gue only. Subsequent collections from Oax have been obtained (*Roe 554*, WIS; *Turner 80A*, TEX).

OTOPAPPUS MICROCEPHALUS Blake, J. Bot. Brit. & For. 53: 232. 1915.

Nay, Jal, Col, Mic and Gue, tropical deciduous forests, Pacific slopes, 5-800 m; Aug-Dec. **Map 3**

Shrub or scrambling vine to 4 m high; leaves 5-12 cm long, 3-5 cm wide; petioles 3-8 mm long; blades ovate to ovate-lanceolate, 3-nervate from the base, sparsely pubescent with appressed hairs beneath, the margins serrulate to nearly entire; heads radiate, 15-70 in terminal corymbose panicles; involucre mostly 3.5-5.0 mm long, 4-6 mm wide, the bracts 3-4 seriate, graduate, the apices obtuse; ray florets 8-13, the ligules yellow, 1.5-3.0 mm long; disk florets 30-75, the corollas yellow; achenes 2-3 mm long, the pappus of 2 unequal awns, 0.3-2.5 mm long.

Hartman and Stuessy (1983) do not report collections from Nay or Mic; several recent collections have been obtained from these states. **Otopappus microcephalus** is similar to **O. serboanus** and **O. tequilanus**, the latter differing mostly by its longer rays, head size and more numerous florets.

OTOPAPPUS ROBUSTUS Hemsl., Biol. Centr. Amer. Bot. 2: 191. 1881.

Zexmenia robusta (Hemsl.) O. Hoffm.

In Mexico, known only from the type locality, vicinity of Cordoba, Ver, ca 1000 m; Mar. **Map 3**

Shrub or clambering vine; leaves 14-24 cm long, 5-8 cm wide; petioles 15-20 mm long; blades ovate, pinnately veined, strigillose above, tomentose beneath, the margins serrulate; heads eradiate, both terminal and axillary, forming a leafy terminal corymbose panicle, the ultimate peduncles 1-5 mm long; involucre 6-7 mm high, 8-10 mm wide, the bracts 4-5 seriate, graduate; rays absent; disk florets 35-45, the corollas yellow, 3-4 mm long; achenes 2-5 mm long, the pappus of 2 winged awns, 1.5-3.0 mm long, between these a crown of scales ca 1.3 mm long.

A poorly known species readily distinguished by its eradiate yellow heads.

OTOPAPPUS SCABER Blake, Contr. U.S. Natl. Herb. 22: 636. 1924.

Cps, Cam and adjacent Guatemala southwards, tropical deciduous forests, 100-1300 m; Oct-Dec. **Map 3**

This species resembles **O. verbessinoides** but the leaves are broader and coarsely hispidulous on both surfaces, and the heads broader with more numerous florets (80+ vs 30-70).

OTOPAPPUS SERBOANA B.L. Turner, *sp. nov.* **Fig 1**

Oax, coastal areas, 10-300 m, localized tropical deciduous forests; Aug-Oct. **Map. 3**

Shrubs, or clambering vines in trees up to 8 m high. **Stems** (upper) pubescent with minute, appressed, upswept hairs. **Leaves**, 6-14 cm long, 3-6 cm wide, opposite throughout, reportedly reflexed; petioles 0.6-1.2 cm long; blades ovate, 3-nerved from near the base, moderately pubescent above and below with short, mostly erect, broad-based hairs. **Capitulescence**, a terminal array of 3-10 heads, the ultimate peduncles 0.5-2.0 cm long. **Heads**, ca 8 mm high and as wide. **Involucres**, ca 4 mm high, 7 mm wide, broadly campanulate. **Involucral bracts**, 4-5 seriate, graduate, broadly ovate, their apices reflexed at maturity, appressed-pubescent throughout. **Receptacle** convex, ca 4 mm across, the pales numerous, linear lanceolate, persistent, 5-6 mm long, ca 0.6 mm wide, their apices sharply acute and somewhat reflexed. **Ray florets** pistillate, fertile, numerous (30 +); ligules yellow, ca 3 mm long, 0.7 mm wide. **Disc florets**, yellow, numerous (80 +); corolla ca 4 mm long, glabrous; tube ca 1 mm long, grading into the throat; lobes ca 0.5 mm long. **Anthers** brown, their apical appendages ovate, glandless. **Achenes** (immature), ca 1.5 mm long, glabrous; pappus a prominently winged awn ca 2 mm long.

TYPE: MEXICO. OAXACA: Distrito Pochutla; Mpio. San Pedro Huamelula, "Desviacion a Chacalapa, 300 m al N rumbo a San Isidro Chacalapa." ca 160 m; 15 52 41 N, 95 56.9 W, 30 Oct 2000, *Silvia H. Salas M. 3448* [with M. Elorsa C. & A. Sanchez] (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. OAXACA. Distrito Pochutla; Mpio. San Pedro Pochutla, "Coastal Region of Oaxaca along a dirt road to Tahueco, ca. 1 km SW of Hwy 200, 11 km ENE of Puerto Angel." 129 m, "Dense tropical deciduous forest." 3 Aug 2003, *Salvato 349* (TEX). **Mpio. Santa Maria Huatulco**, "160mts.[sic] (L.R.) 178 grados de la entrada hacia la laguna del zanate sobre el Sendro del Caminante." 15 43 59.7 N, 96 09 15.1 W, ca 10 m, 29 Oct 2004, *Martinez 228* (TEX).

The Martinez and Salvato specimens, cited above, are very immature (lacking well-defined rays or disc florets), and when initially examined I took these to be *Otopappus microcephalus*; subsequent examination of the Type of *O. serboana*, possessing flowering heads, showed the plants to have a number of features that distinguished the taxon, as noted below. The sterile plants clearly belong to the novelty described here, having most of its features.

Salvato notes the plant to be an "Uncommon rambling shrub ca 4 ft tall."

Otopappus serboana is clearly related to *O. microcephalus*, occupying a similar shore line habitat, but is readily distinguished from the latter by its mostly larger, fewer, heads and more numerous ray and disc florets.

The species is named for the organization of SERBO, which funded its collection.

OTOPAPPUS TEQUILANUS (S. Wats.) B.L. Rob., Proc. Amer. Acad. Arts 44: 622. 1909.

Notoptera tequilanus (S. Wats.) Blake

Otopappus salazari Blake

Otopappus tequilanus var. *griseus* McVaugh

s Sin, Zac, Nay, Jal, Col, Mic, Mor, Pue and Gue, tropical deciduous and pine-oak forests, mostly Pacific slopes, 100-1300 m; Aug-Nov. **Map 4**

Vegetatively and in vestiture, much resembling *O. microcephalus*, but in head size, floret number and ray length much closer to *O. acuminatus*; chromosome number, $n = 16$ pairs.

Hartman and Stuessy (1983) did not recognize the var. *griseus* but McVaugh (1984) retained the taxon, distinguishing this largely by its leaves, which were said to be "silvery white beneath with closely aggregated stiff appressed hairs and very fine cottony hairs." He noted that the characteristic gray vestiture of the leaves may be partly due to a fungal infection. On total characters, however, the variety appears too weakly differentiated for recognition.

OTOPAPPUS VERBESINOIDES Benth., Hooker's Icon. Pl. 12: 47. 1873.

Otopappus trinervis Blake

Ver, Oax, Cps and Guatemala southwards, tropical evergreen cloud forests, 20-2100 m; Aug-Feb.
Map 4

Shrubs or clambering woody vines to 12 m high; leaves 7-16 cm long, 1.5-5.0 cm wide; petioles 3-10 mm long; blades narrowly ovate, attenuate apically, 3-nervate and reticulate-veined beneath, strigillose with appressed hairs, the margins serrulate; heads radiate, 3-10 in terminal or subterminal cymose clusters; involucre campanulate 4-6 mm high, 5-11 mm wide, the bracts 4-5 seriate, the outer series green and loose, oblanceolate, often longer than the inner bracts; ray florets 8-15, pistillate, fertile, the ligules 3-15 mm long, yellow; disk florets 30-70, the corollas yellow, achenes 3-5 mm long, the pappus of a single awn 2.5-3.0 mm long, the scales united into a crown 1.0-1.5 mm long.

ACKNOWLEDGEMENTS

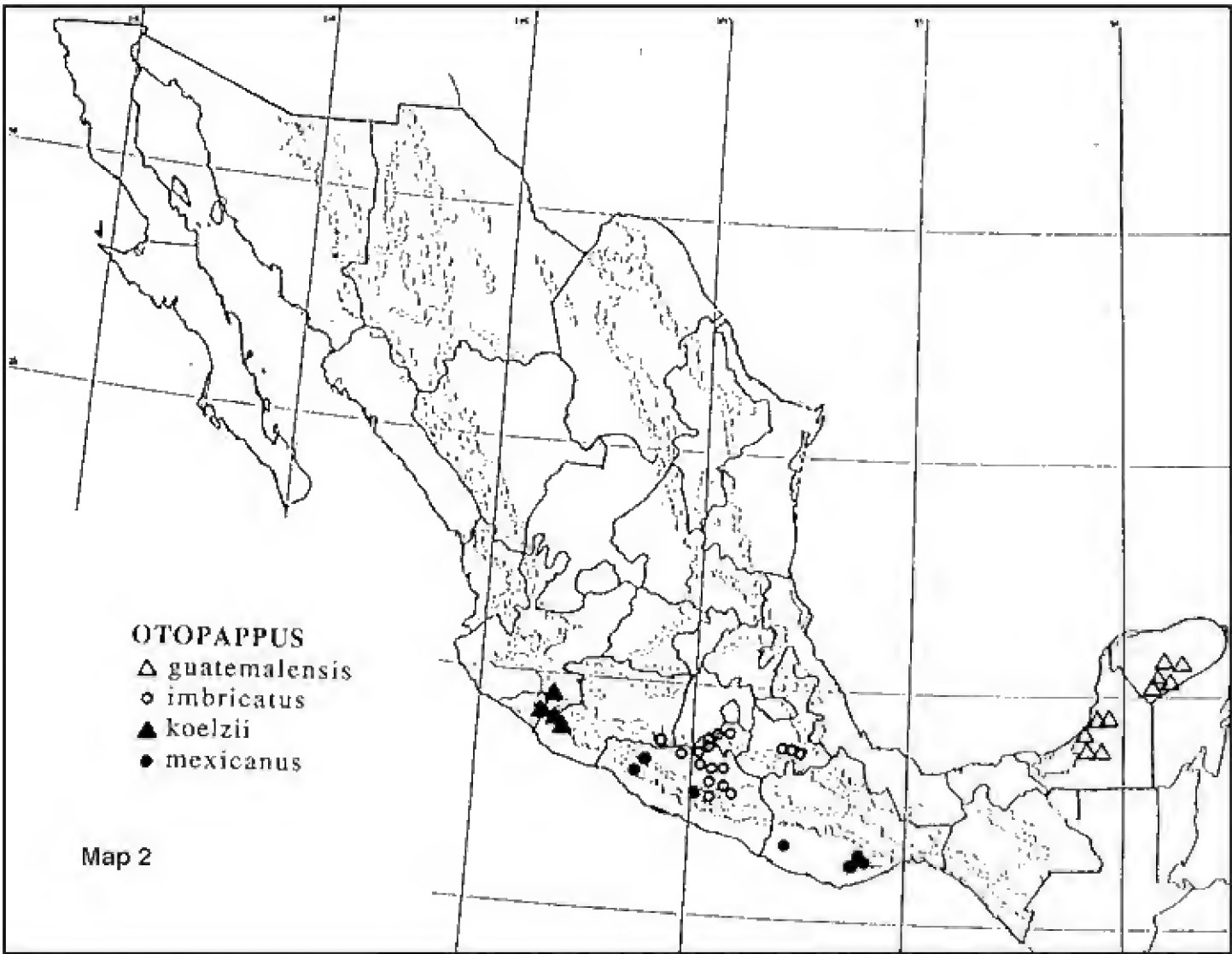
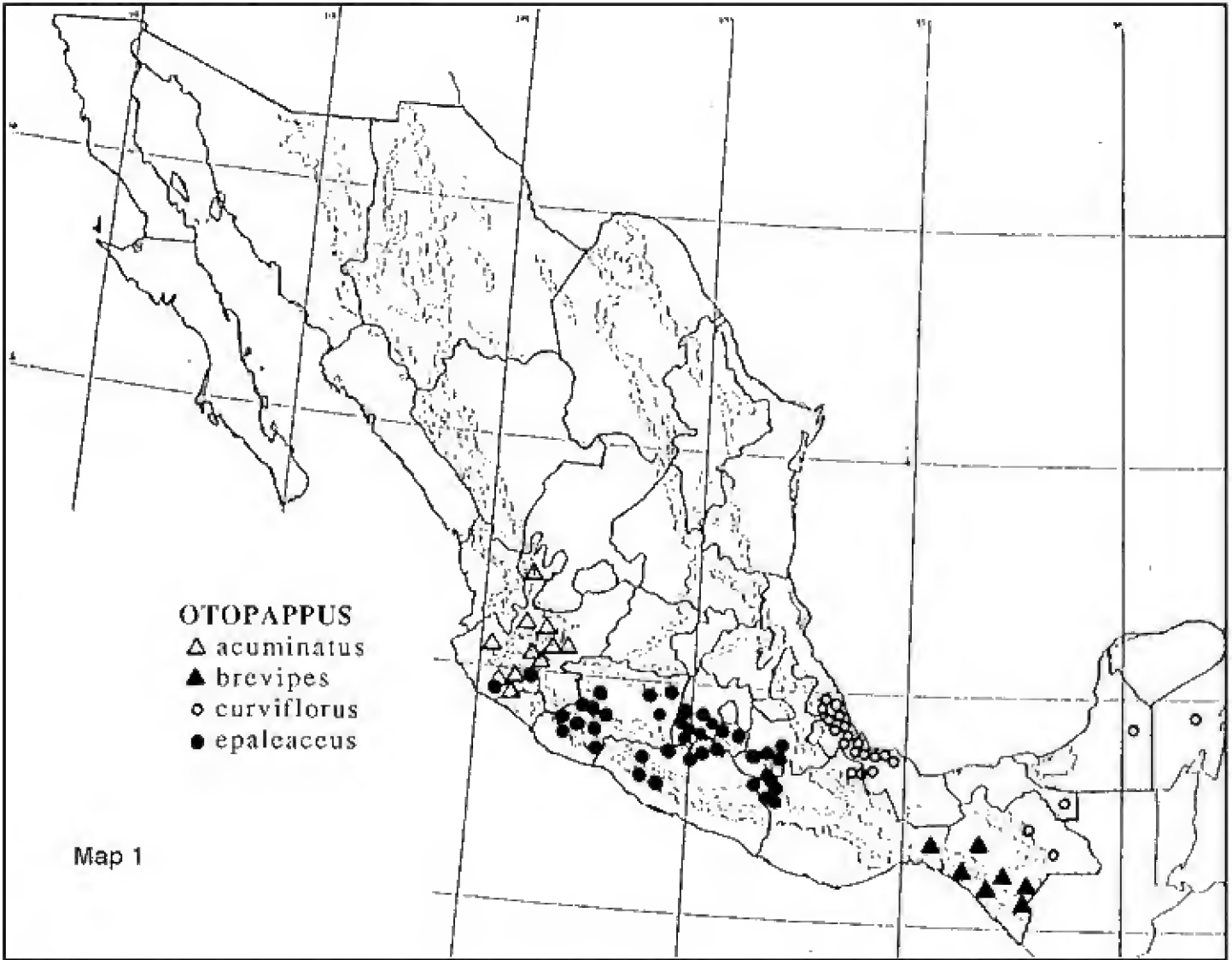
The anagrammatic organization, SERBO, provided plants of **O. serboanus** for identification. My intellectual companion, Jana Kos, provided editorial skills, for which I am grateful. Distribution maps are based largely upon collections at LL-TEX.

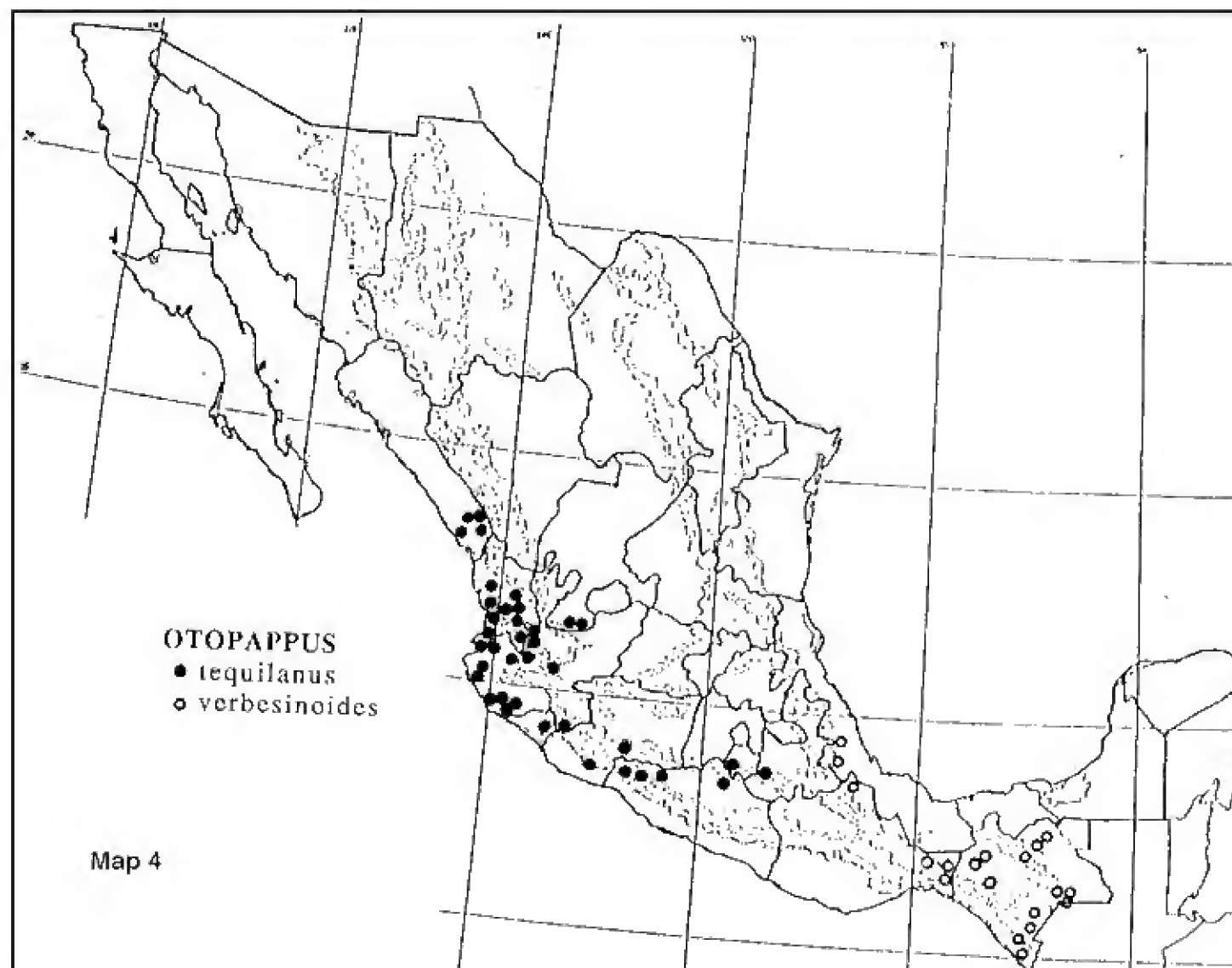
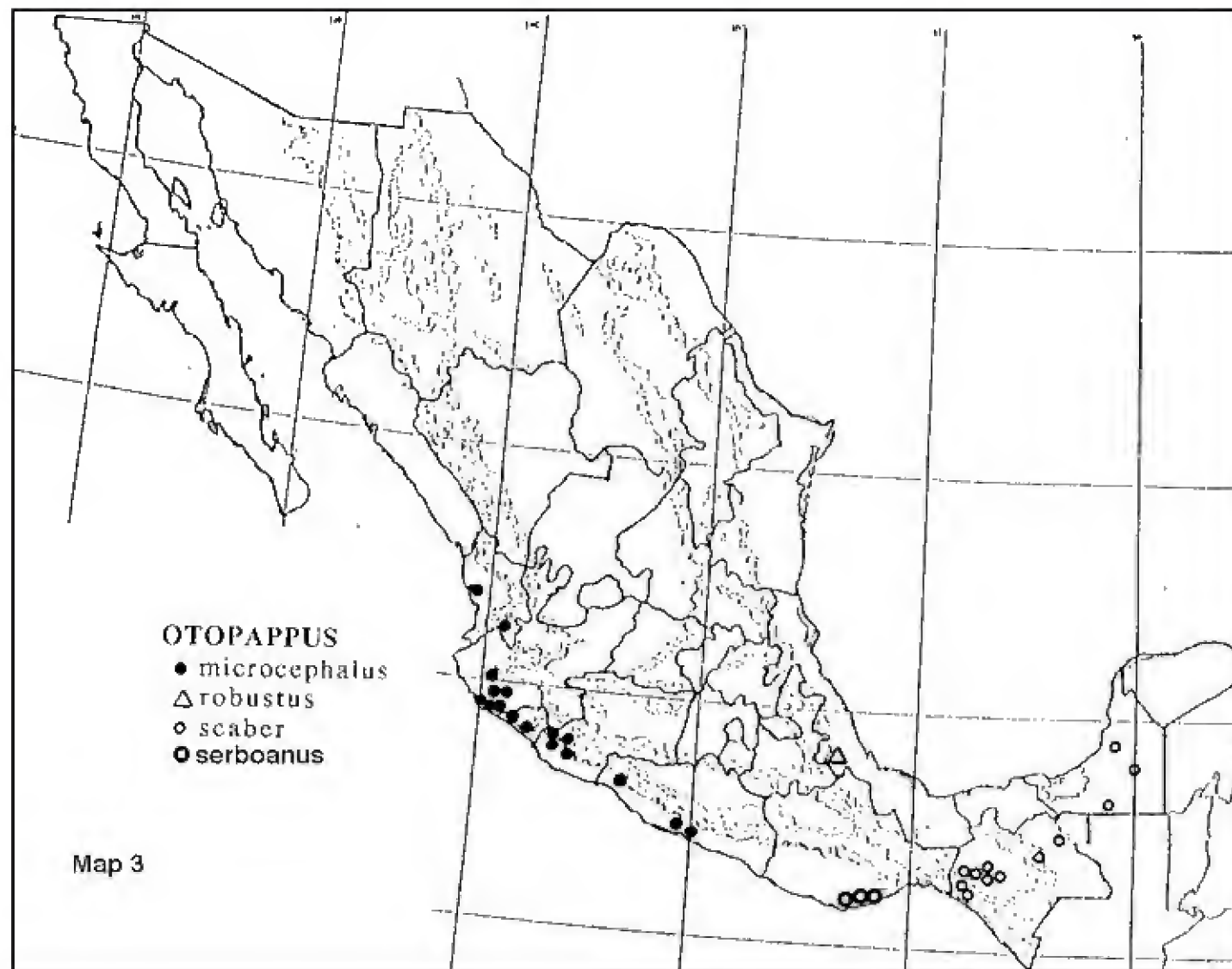
LITERATURE CITED

[See **REFERENCES** just after the generic description, above.]



Fig. 1. OTOPAPPUS SERBOANA B.L. Turner, sp. nov.





Taxonomy of *Juniperus deppeana* varieties and forms based on nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF sequences

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ABSTRACT

Juniperus deppeana has numerous disjunct populations that include four taxonomic varieties and three forms. All four varieties and two forms of *Juniperus deppeana* from the southwest United States, Mexico and Guatemala were analyzed by sequencing nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF. A Bayesian tree gave support for clades of var. *deppeana*, NM, var. *patoniana*, and var. *gamboana*. However, several clades with high support contain mixtures of different varieties and forms. A minimum spanning network based on 91 mutational events (MEs) showed that the varieties and forms are extremely closely related, differing by only 1 to 2 bp (out of 4411 bp). The taxon with the largest differentiation was var. *deppeana*, Sacramento Mtns., NM that differed by 4 MEs from the Oak Creek canyon, AZ individuals. The lack of variation among *J. deppeana* taxa may be due to the mixing of populations during the Wisconsin glacial maximum (70,000 - 13,000 ybp) when life zones descended about 800 m. Published on-line: www.phytologia.org *Phytologia* 95(2): 161-166 (May 1, 2013).

KEY WORDS: *Juniperus deppeana* varieties, Cupressaceae, DNA, nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, systematics, geographic variation, taxonomy.

Juniperus deppeana Steudel has trunk bark that exfoliates in quadrangular plates, thus the common name 'alligator bark' juniper. *Juniperus deppeana* is part of the serrate leaf margined species of the western hemisphere (Adams, 2011) and is widely distributed in the southwestern US, Mexico and northern Guatemala (Fig. 1). Putative *Juniperus d. f. sperryi*, once known only from the type locality in the Davis Mtns., TX, has been found in Arizona and New Mexico (Fig. 1). However, the bark characters seem to be controlled by only a few genes thus furrowed bark may have arisen independently in western Mexico and the southwestern United States (e.g., *J. d. var. patoniana* and *J. d. f. sperryi*). Whether the furrowed bark trees of Mexico are related to *J. d. f. sperryi* is not well understood.

The first systematic treatment of the serrate leaf-margined junipers was by Martinez (1963) who recognized *J. deppeana* Steudel. var. *deppeana* (checkered bark, (3)4-5(6) seeds/cone,

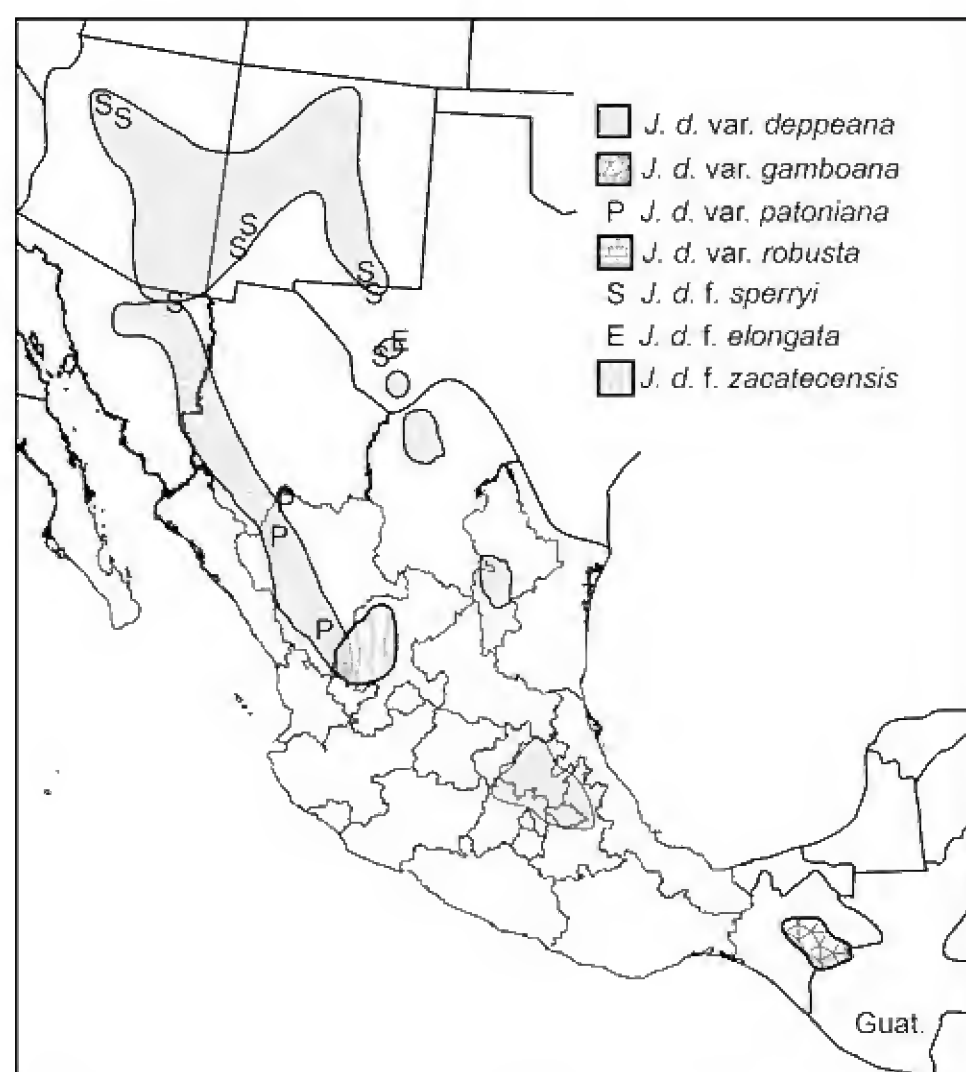


Figure 1. Distribution of *J. deppeana*.

J. d. var. pachyphlaea (Torrey) Mart. (checkered bark, (1)2-4(5) seeds/cone), *J. d. var. robusta* Mart. (checkered bark, (1)2-3(-6) seeds/cone), *J. d. var. zacatecensis* Mart. (checkered bark, 1-4(-7) seeds/cone), *J. patoniana* Mart. (laced bark, (1)2-3(-6) seeds/cone, and *J. gamboana* Mart. (checkered bark, 1(2) seeds/cone) (Fig. 2).

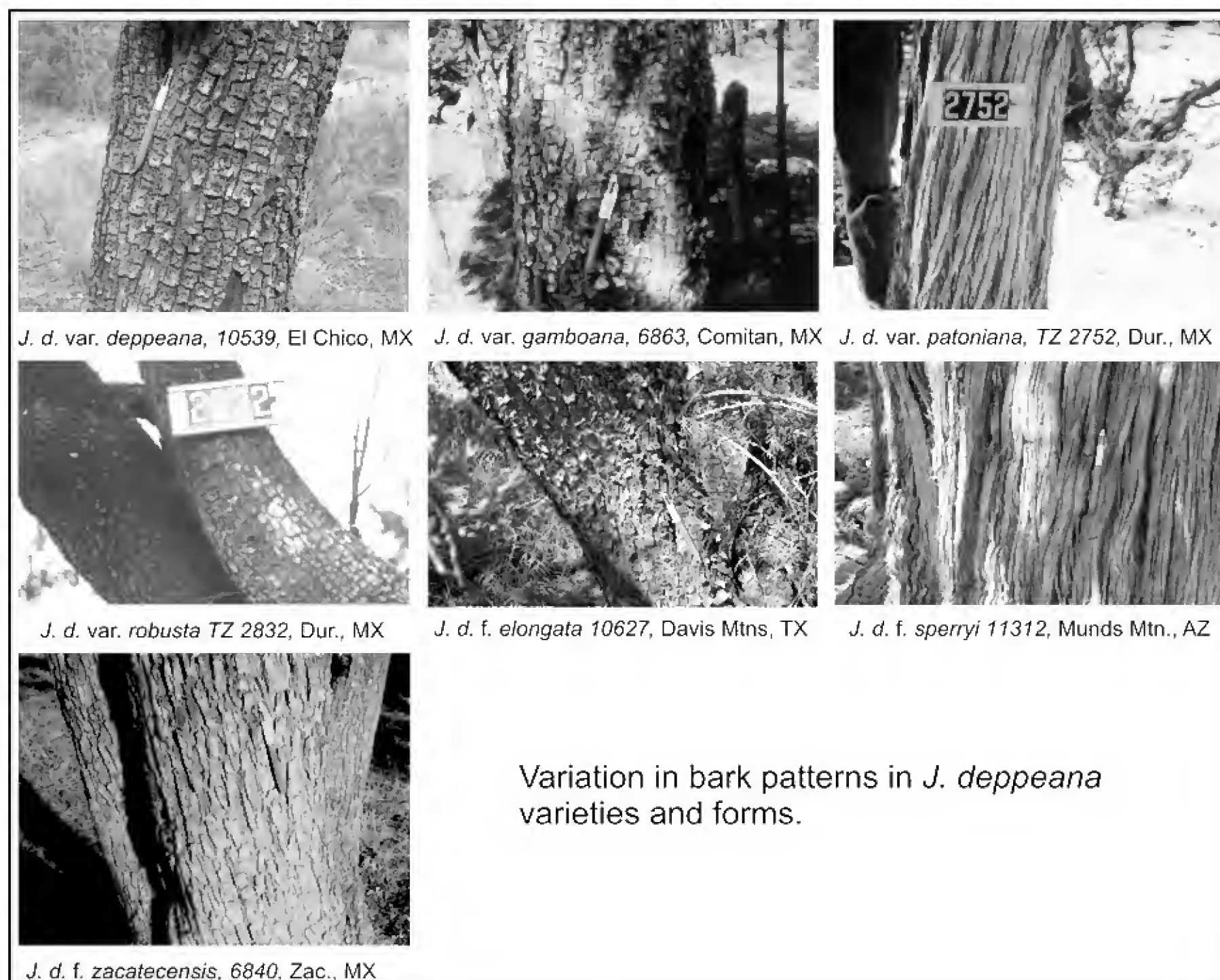


Figure 2. Variation in bark exfoliation among *J. deppeana* varieties and forms.

Zanoni and Adams (1976, 1979) and Adams, Zanoni and Hogge (1984), using morphology and essential oils, generally agreed with Martinez's treatment, except *J. patoniana* was reduced to *J. d. var. patoniana* (Mart.) Zanoni. Additional studies (Adams and Nguyen, 2005; Adams et al. 2007) have further clarified geographical variation in *J. deppeana*.

Recently, Adams and Schwarzbach (2006) recognized *J. gamboana* as *J. deppeana* var. *gamboana* (Mart.) R. P. Adams and *J. deppeana* var. *zacatecensis* as *J. deppeana* f. *zacatecensis* (Mart.) R. P. Adams. Adams and Schwarzbach (2011) found *J. deppeana* and var. *gamboana* to be a clade, sister to *J. ashei*, *J. saltillensis* and *J. zanonii* (Fig. 3). However, the other *J. deppeana* varieties and forms were not included in their study.

The focus of the present study was to examine relationships among all the recognized (Adams 2011) varieties and forms of *J. deppeana* (except the very minor variant f. *elongata*, Adams 2011) using data obtained from sequencing of nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF.

MATERIALS AND METHODS

Specimens used in this study: *J. deppeana* var. *deppeana*, Adams 10539-10541, El Chico National Park, Hidalgo, MX; Adams 7632-7634, Sacramento Mtns., e of Alamogordo, NM, USA; Adams 10640-10642, Oak Creek Canyon-Flagstaff, AZ; *J. deppeana* var. *gamboana*, Adams 6863-6867, Comitán, Chiapas, MX; *J. deppeana* var. *patoniana*, Adams 6836-6839, km 152, w. of Durango (city), Durango, MX (P); *J. deppeana* var. *robusta*, Adams 10255-10256, w of La Ciudad, Durango, MX; *J. deppeana* f. *sperryi*, Adams 10626, Bridge Spring, Davis Mtns., TX, USA; Adams 11312, Munds Mtn., AZ; *J. deppeana* f. *zacatecensis*, Adams 6840-6842, 18 km w. Sombrette, Zacatecas, MX; *J. virginiana*, Adams 10231-10232, Knoxville, TN, USA. Voucher specimens are deposited at BAYLU herbarium, Baylor University.

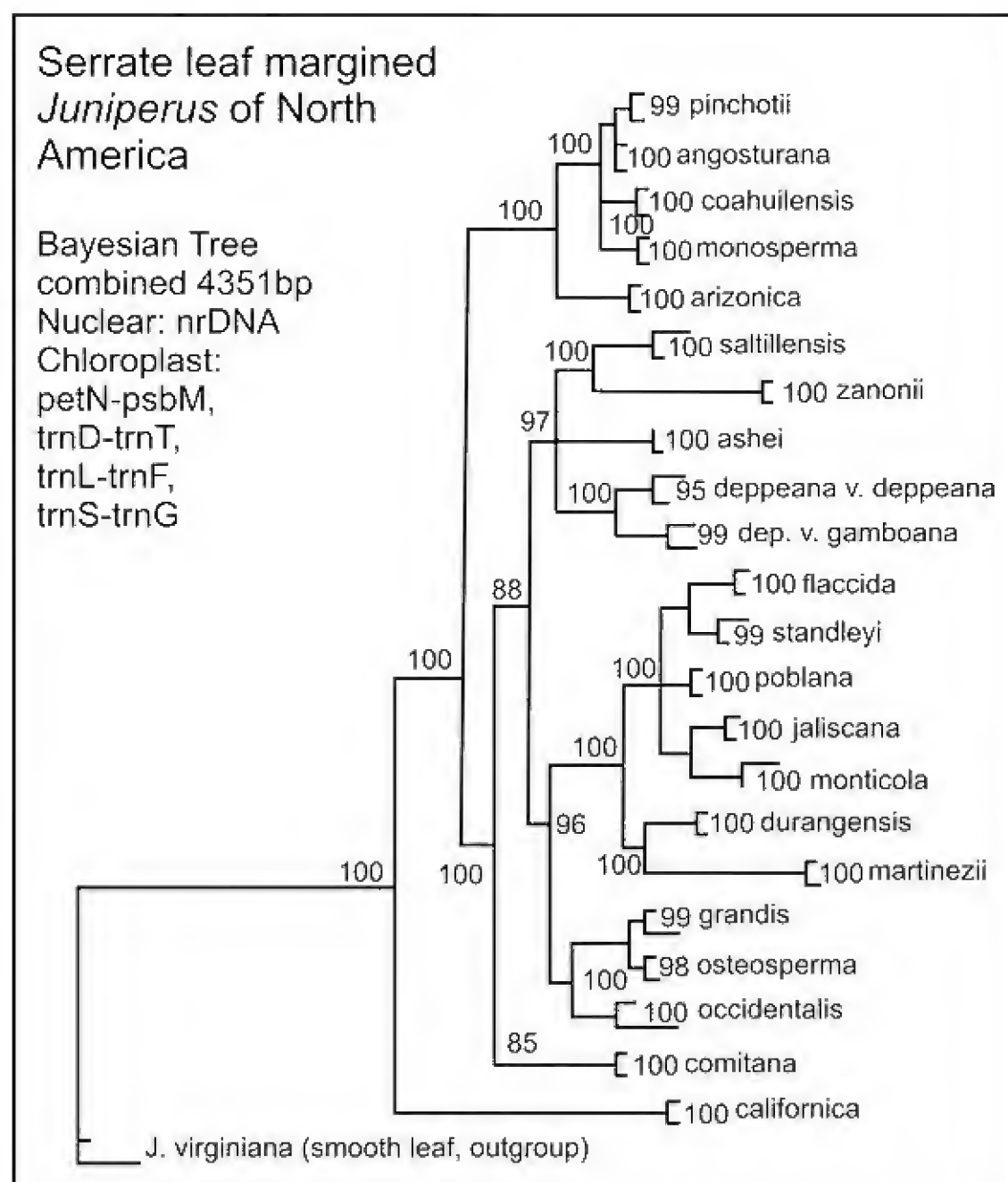


Figure 3. Bayesian tree of the serrate *Juniperus* of North America. Numbers at the branch points are posterior probabilities (as percent).

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20°C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 μl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 μl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 μM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl_2 according to the buffer used) 1.8 μM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. 5.4 (Drummond et al. 2011), the MAFFT alignment program and the PAUP* program, version 4.0b10 (Swofford 2003) for neighbor joining, parsimony, and maximum likelihood tree searches. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1

(Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing the five gene regions (nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF) resulted in 4411 bp of data. A Bayesian tree based on these data (Fig. 4) supports clades of var. *deppeana*, NM, var. *patoniana*, and var. *gamboana*. However, several clades with high support contain mixtures of different varieties and forms. Note that accessions of var. *robusta* are in different clades, as are the samples of f. *sperryi* from AZ and TX. It should be noted that hybridization between varieties and forms should be expected as these dioecious taxa are out-crossing plants in populations where several infraspecific taxa are often present.

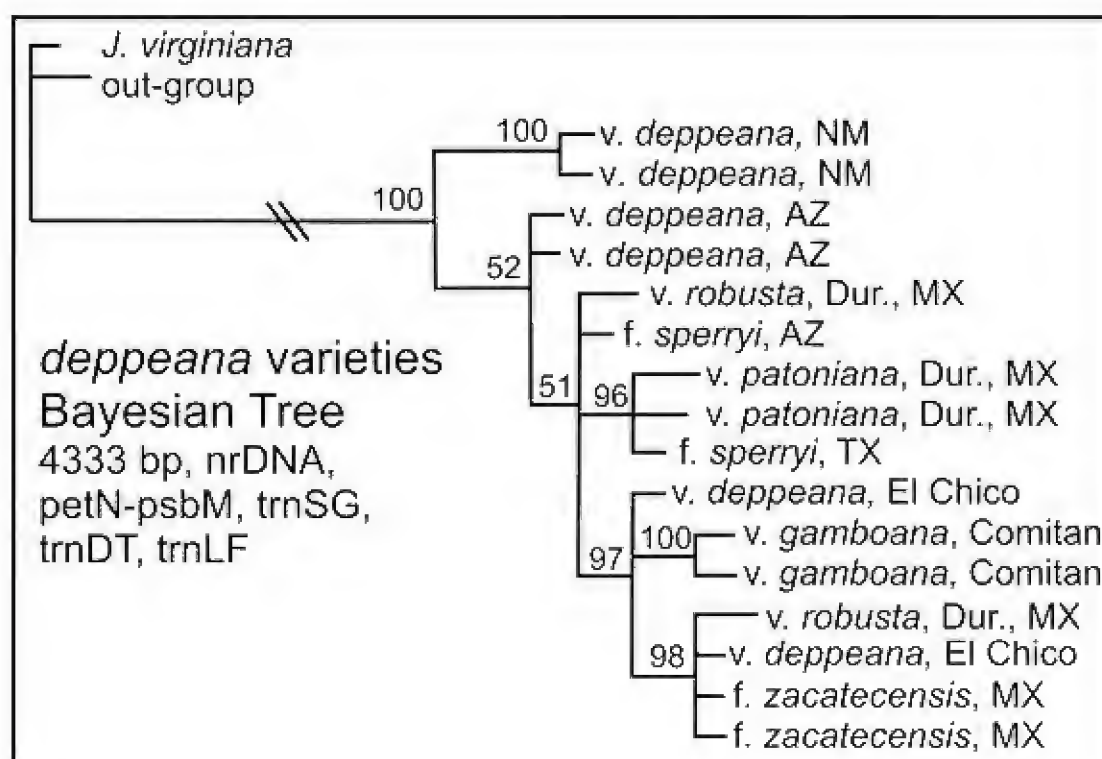


Figure 4. Bayesian tree of *J. deppeana* varieties. Numbers at branch points are posterior probabilities (as percent).

Although the Bayesian tree indicates high support for some clades (Fig. 4), the magnitude of differentiation among accessions is not apparent. A minimum spanning network based on all mutational events (MEs) assimilates both the nucleotide substitution and indel information. This analysis revealed 97 MEs, with 6 MEs found only once, and 91 MEs found multiple times. Of the 91 MEs, 70 differentiated *J. virginiana* from *J. deppeana* (Fig. 5). Thus, the entire differences among these 4 varieties and 2 forms amount to only 21 MEs. In general, only 1 or 2 MEs separate individuals (Fig. 5). The exception is the differentiation of plants from NM (Fig. 4) that are separated from the AZ plants by 4 MEs.

Adams and Schwarzbach (2012) found that traditionally recognized taxonomic species differed by 8 to 12 (or more) MEs, whereas varieties appeared to differ by less than 8 MEs. The differences between var. *deppeana* from AZ and NM, although interesting, do not appear to be

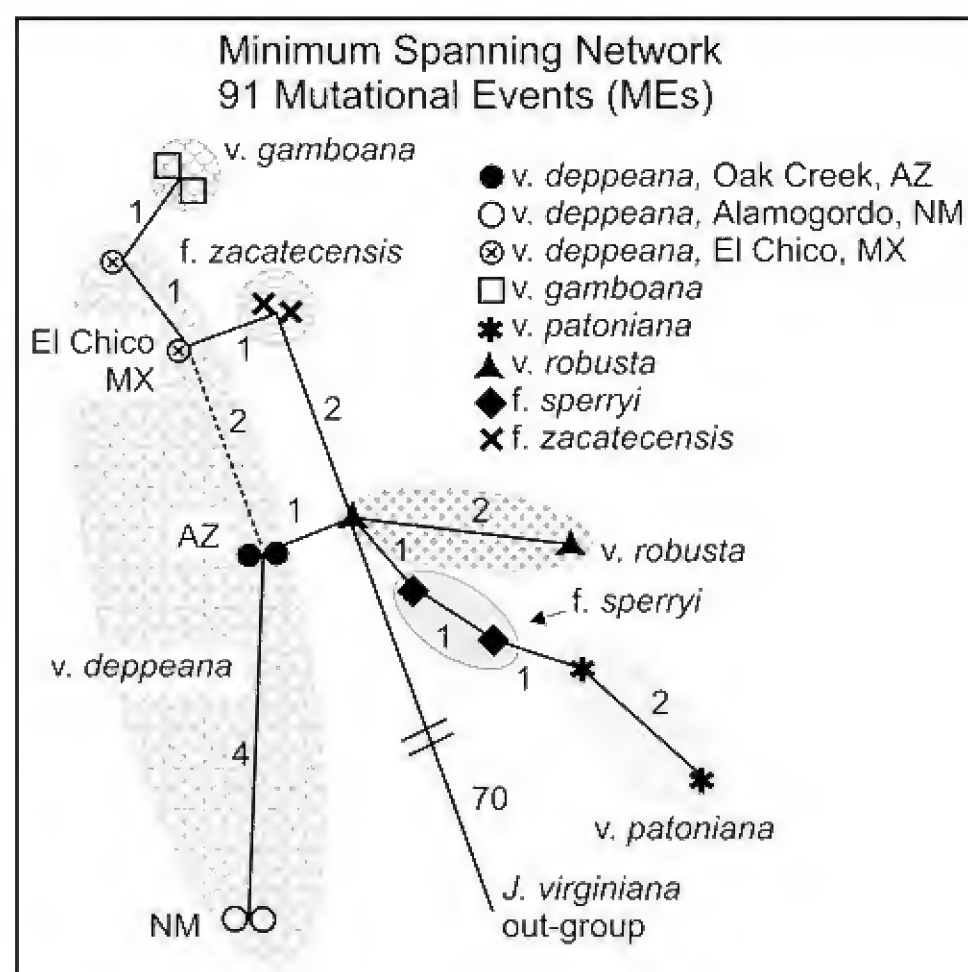


Figure 4. Minimum spanning network. Numbers on the lines are the number of MEs.

sufficient, by themselves, to warrant the recognition of a new variety from NM. Additional research on geographic variation in leaf terpenes and morphology is in progress so as to examine differentiation in the southwestern US.

CONCLUSION

The mixtures of various taxa within clades may be due to ancient climate and past distributions of *J. deppeana*. Wells (1966), using data from rat middens from the Big Bend of Trans-Pecos, Texas, concluded that during the Wisconsin (70,000 - 13,000 ybp) life zones descended about 800 m leading to the formation of a pinyon-juniper woodland in the present Chihuahuan desert between the Big Bend of Trans-Pecos, Texas and the city of Del Rio. Assuming that the effects of glaciation were mediated southward into Mexico so that life zones descended only a few hundred meters in Hidalgo, it appears that most of the now disjunct populations of *J. deppeana* may have once been connected in a nearly continuous population of distribution around the Chihuahuan desert (Fig. 5). It is likely that desert peaks within the area concerned also supported stands of *J. deppeana*. Wisconsin populations would have become spatially separated as dryer, warmer climate developed during the Holocene (past 13,000 y). Of course, the Wisconsin was only the most recent of several pluvial events during the Pleistocene, spanning 1.8 my (Flint, 1971). It is likely that during any one (or several) of these pluvial events, *Juniperus deppeana* occupied lower elevation and more southward habitats, leading to more contiguous populations in Mexico and the southwestern United States. If divergent populations (or varieties) became sympatric during the Wisconsin, this would have facilitated infra-specific crossing. This may account for the large genetic variation within some populations. In addition, the millennia of continuous populations could explain the lack of differentiation between the recently (Holocene) geographically isolated populations.

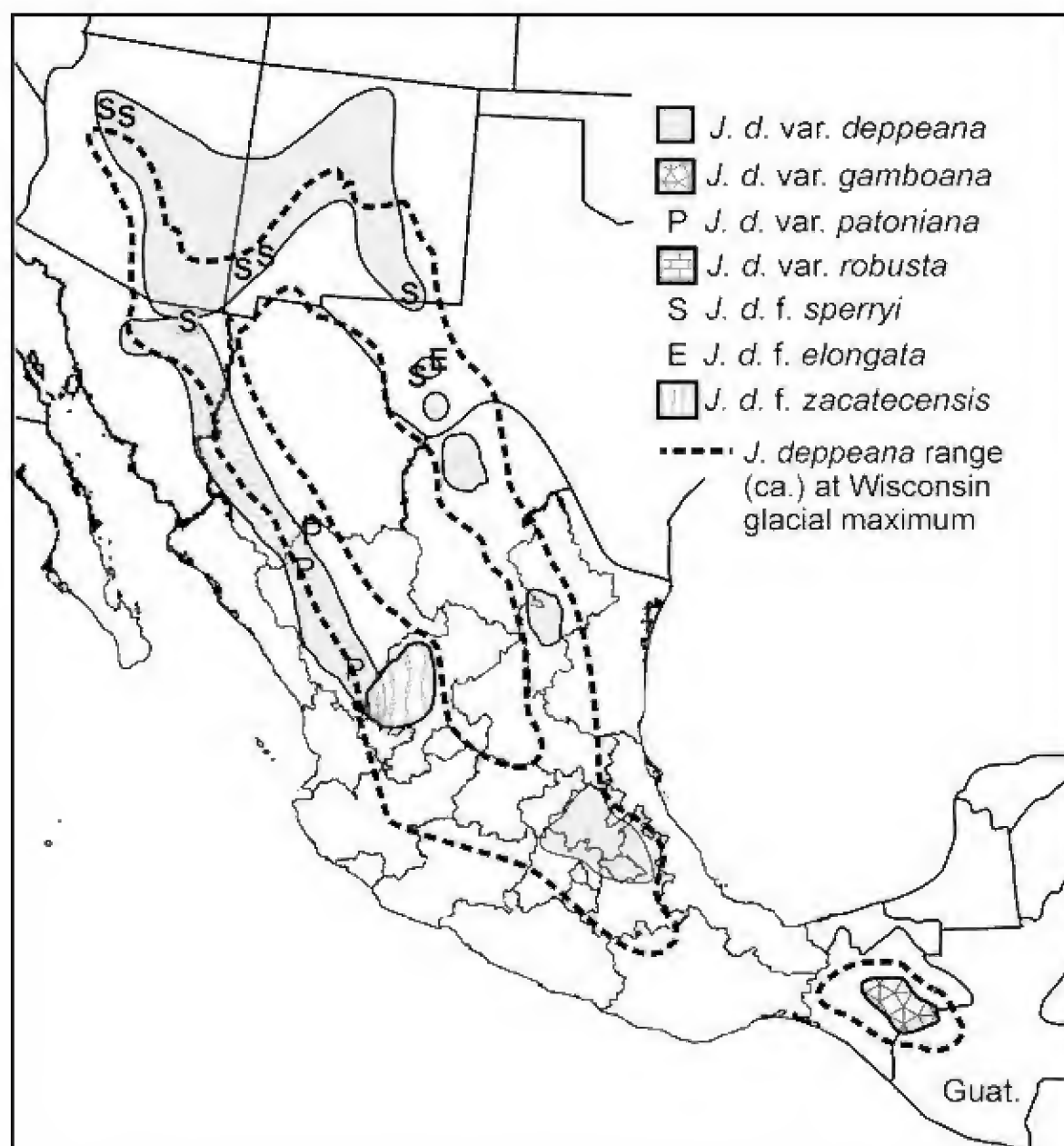


Figure 5. Possible range of *J. deppeana* during the Wisconsin glacial maximum (based on Wells, 1966). The present day disjunct populations were likely continuous in the foothills around the Chihuahuan desert during the Wisconsin.

ACKNOWLEDGEMENTS

Thanks to Tom Zanoni (NYBG) for providing slides of tree barks and assistance in field work, and Tonnie Yanke for lab assistance. This research was supported in part with funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

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***Jaltomata spooneri* (Solanaceae): A new species of Southern Peru**

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ABSTRACT

A new species having edible fruit, *Jaltomata spooneri* Mione & S. Leiva (Solanaceae), is described from Peru, Departments Puno and Cuzco, and shown in photographs. Flowers are protogynous and stamens elongate after the flower opens. *Jaltomata spooneri* is similar to *J. contumacesis* S. Leiva & Mione, *J. sanchez-vegae*, S. Leiva & Mione and *J. yacheri* Mione & S. Leiva, also restricted to Peru. Differences among these species are shown in a table. www.phytologia.org *Phytologia* 95(2): 167-171 (May 1, 2013).

KEY WORDS: Andes, Cuzco, edible fruit, *Jaltomata spooneri*, Peru, Puno, Solanaceae

This paper is a contribution to ongoing taxonomic studies of the genus *Jaltomata* (Mione et al., 2007; Leiva, et al., 2010; Mione et al., 2011). During fieldwork in 2010 we collected the following new species.

JALTOMATA SPOONERI Mione & S. Leiva sp. nov. TYPE: PERU. PUNO. Prov. Sandia, Patambuco 14° 21' 41.5" S, 69° 37' 18.4" W, 3,600 m, 9 Jan 2010, *T. Mione, S. Leiva G. & L. Yacher et al.* 799 (holotype: HUSA; isotypes: F, NY). Figures 1- 5.

Shrub to 2 m high; young axes green, angular, sparsely hairy with dendritic and uniseriate unbranched (finger) hairs, some gland-tipped; older stems woody, terete, with lenticels. Leaves alternate, often geminate, the blade papyraceous, to 8.6 X 11.7 cm long, lanceolate or ovate with the apex acuminate, minutely pubescent on both faces, the hairs simple and / or dendritic, the margin entire to somewhat repand, ciliate; petiole to 2.7 cm. Inflorescence axillary, 2-(3) flowered and fruited; peduncle to 15 mm, terete, green; pedicel 7—20 mm, green, with 5 raised longitudinal ridges. Calyx green, rotate during anthesis (Figures 1 & 3), to 23 mm across, the lobes triangular, adaxially glabrous, abaxially with a mixture of gland-tipped finger and forked hairs; calyx with immature fruit to 25 mm across (Figure 3). Corolla urceolate-tubular with a broad, recurved 10-lobed limb, light-green to whitish, 1.5—2.0 cm long, abaxially sparsely pubescent with finger hairs occasionally gland-tipped, the limb 2.5 cm across, with 5 cream-green, narrowly triangular lobes alternating with 5 lighter lobules, the margin ciliate (Figure 1). Stamens to 27 mm, the filaments unpigmented, glabrous, elongating while flower is open; anthers 2.1 — 2.7 mm when dehiscent and pressed, mucronulate, exerted beyond the mouth of the corolla. Radial expansions of the bases of the stamens, adnate to the corolla, create nectar troughs between the radial expansions (Figure 2). Pollen grains 33 — 38 µm diameter (n = 18 grains, mean 35 µm), 92,500 — 109,000 per androecium (n = 2 flowers). Style 26—28 mm (Figure 3), pale green; stigma capitate, bilobed (sometimes obscured by pressing), darker green than style, exerted 0 — 2 mm beyond the dehiscent anthers; gynoecium glabrous, the disk girdling the base of the ovary (Figure 4), the nectar

translucent. Berries subspherical (Figure 3), most likely orange, and to 13 mm across on herbarium specimens; seeds light brown, sub-reniform to sub-orbicular.

Specimens Examined. **PERU. CUZCO: Prov. Quispicanchi**, Marcapata, 15-16 Feb 1929, *Weberbauer* 7789 (NY, US); 3,100 m, 7 Dec 1962, *Vargas C.* 14039 (CUZ); **PUNO: Prov. Carabaya**, 3,425 m, 31 Dec 1947, *Vargas C.* 6978 (CUZ, US); 2,800 m, 17 Feb 1983, *Ochoa & Salas* 15075 (US); 3,300-3,700 m, 5 Mar 2004, *Ortiz V. et al.* 5 (HUSA); 3,300-3,500 m, 6 Mar 2004, *Vilca C. et al.* 45 (HUSA); La Escalera / Kana, 3,598 m, 10 Jan 2010, *Mione et al.* 800 (F), *Leiva G. et al.* 4656 (HAO); **Prov. Macusani**, road from Ollachea to Macusani, 13° 50' S, 70° 29' W, ca 3,200 m, “1.2.2000,” *Weigend* 2000/100 (HUSA); **Prov. Sandia**, Limbani, 3,000 m, 22 Nov 1938, *Vargas C.* 1299 (CUZ, MO); 3,400 m, 21 Nov 1938, *Vargas* 9654 (G); El camino de Machu Tticani a Patanbino, 3,300 m, 19 Feb 1983, *Ochoa & Salas* 15085 (US); from Patambuco 3.6 km out of town to Escuela San Luis, ca 300 m from school along path to valley of Río Rumichaca, 14° 23' S, 69° 36' W, 3,500 m, 27 Feb 1998, *Spooner et al.* 7402a (herbarium of Thomas Mione); Same location and date as type collection, *Leiva G. et al.* 4655 (HAO).

Discussion. *Jaltomata spooneri* grows in Peru, in the departments of Cuzco (province Quispicanchi) and Puno (provinces Carabaya, Macusani and Sandia). Specimens were collected between 2,800 and 3,700 m. The habitat was described by collectors as occurring close to fences, dwellings, hedges, roadsides, and rock walls near cultivated land or in cloud forest remnants. Flowers are apparently protogynous, and stamens elongate after the flower opens: on the same plant we saw open flowers having undehiscent anthers on shorter filaments, and flowers with dehiscent anthers on longer filaments (Figure 1). In the population where the type specimen was collected the corolla is light-green to whitish, but in another population (*Mione et al.* 800) the corolla has purple longitudinal veins (Figure 3). The fruits are eaten by local people (Figure 5; *Vargas C.* 1299, *Vargas* 9654, *T. Mione et al.* 800). Local names are “Ahuaimantu” (*Vargas C.* 1299), “Aguaymantai” (*Vargas* 9654) and “Chilto” (type collection).

Jaltomata spooneri is most similar to *J. contumacensis* S. Leiva & Mione, *J. sanchez-vegae* S. Leiva & Mione and *J. yacheri* Mione & S. Leiva, also restricted to Peru. All of these shrubs have 2 — 3 or 2 — 4 flowers per inflorescence, green calyces, urceolate corollas, corolla limbs having lobes alternating with conspicuous lobules, radial thickenings (Figure 3), and yellow anthers. Differences among these species are listed in Table 1.

The specific epithet honors David M. Spooner for his repeated generous gifts of *Jaltomata* seeds and specimens to T. M. for study.

ACKNOWLEDGEMENTS

We thank Gregory J. Anderson and Stacey D. Smith for review, the curators of BH, COLO, F, K, MO, NY and US for loan of specimens, and Emmett P. Varricchio for the pollen count.

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Table 1. Comparison of *Jaltomata spooneri* with similar species.

	<i>J. contumacensis</i>	<i>J. sanchez-vegae</i>	<i>J. spooneri</i>	<i>J. yacheri</i>
Departments in Peru where found	Cajamarca	La Libertad Cajamarca	Puno Cuzco	Cajamarca
Elevation Range, m	2,500 – 3,000	3,000 – 3,550	2,800 – 3,700	3,460 – 3,515
Maximum plant height m	4	1.4	2	1.5
Density of hairs on young axes	Dense to sparse	Dense	Sparse	Dense
Hairs of young axes	Dendritic and some interspersed finger hairs, not-gland-tipped	Finger hairs, all gland-tipped	Dendritic, forked and finger hairs, some gland-tipped, most not	Dendritic hairs, not gland-tipped hairs
Corolla color	Green, sometimes with a purple base	Green with a purple base	Light-green to whitish, sometimes with purple longitudinal veins	Blue-purple
Corolla limb	Nearly rotate	Recurved	Recurved	Recurved
Nectar color	Usually clear, sometimes orange	Usually clear, turning orange	Clear	Clear
Filaments	Pubescent proximally	Pubescent proximally	Glabrous	Pubescent proximally
Length stamens exert beyond mouth of corolla after anthers dehisce, mm	8	15	14	14
Style length, mm	16	17 – 19	26 – 28	15 – 20



Figure 1. Flowers of *Jaltomata spooneri*, type collection. Note protogyny and filament elongation: the shorter stamens have undeveloped anthers while longer stamens have dehiscent anthers. Holes in the bases of the corollas suggest nectar robbing (floral visitors not seen). The corolla is 1.5 to 2 cm long. Photo by Segundo Leiva G.

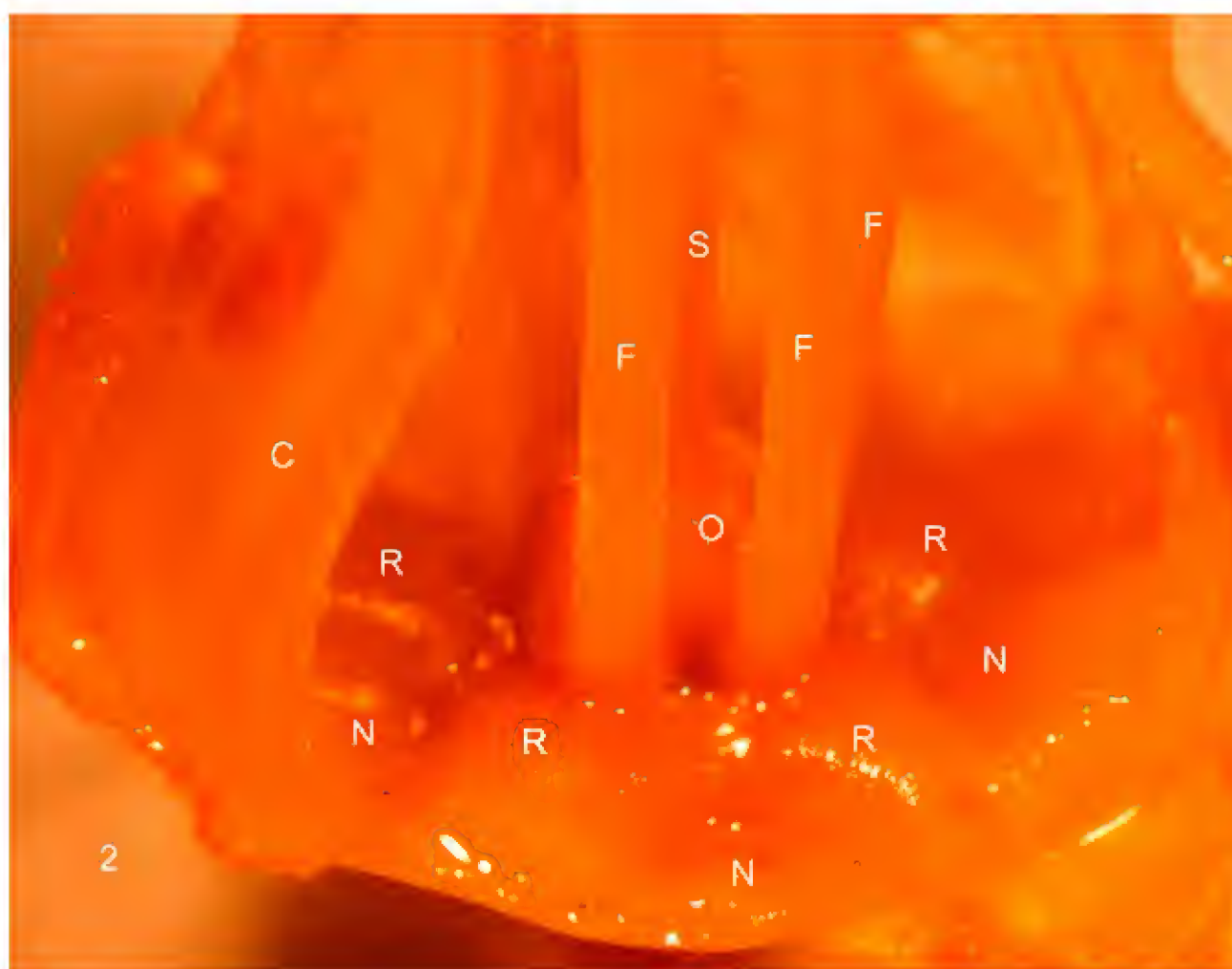


Figure 2. Flower dissected open to show: C, corolla; F, filament; N, nectar troughs between R, radial thickenings; O, ovary; S, style. Colors lost due to preservation in 70% ethanol; photo by Thomas Mione.



Figure 3. The corolla in this population differs from that of the type by having purple longitudinal veins, as seen in the flower on the left (anthers dehiscent). The style and the ovary of a flower can be seen because the corolla-androecium abscised. The fruits are unripe. *Mione et al.* 800; photo by Thomas Mione.

Figure 4. Ovary of flower dissected to reveal ovules (O) and ovarian disk (OD). Ovary 3 mm high X 3 mm diameter at base including ovarian disk. Colors lost due to preservation in 70% ethanol; photo by Thomas Mione.



Figure 5. *Jaltomata spooneri* held by local woman who told us that the fruits of this plant are eaten, “frutilla se comen.” *Mione et al.* 800; photo by Thomas Mione.

**Taxonomy of the serrate leaf *Juniperus* of North America:
Phylogenetic analyses using nrDNA and four cpDNA regions.**

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ABSTRACT

The serrate leaf *Juniperus* of North America were analyzed by nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF sequencing (4411 bp). The varieties of *J. ashei* (var. *ashei*, var. *ovata*) were found to be in separate clades, supporting the recognition of *Juniperus ovata* (R. P. Adams) R. P. Adams, **comb. & stat. nov.** *Juniperus zanonii* has been treated as a *J. monticola* f. *compacta*, but its DNA was very distinct and it was well supported in a clade with *J. saltillensis*, not with *J. monticola*. In the single seeded group, *J. arizonica* was the most distinct species with 8 mutational events (MEs) and *J. angosturana* - *J. pinchotii*, the least distinct (1 ME). Yet, *J. angosturana* and *J. pinchotii* are quite different in their morphology and leaf essential oils (Adams 2011). In the three western US junipers, *J. grandis* was separated by only 4 MEs from *J. osteosperma* and 7 MEs from *J. occidentalis*. The varieties of *J. deppeana* were mostly unresolved showing their close relationship (Adams and Schwarzbach 2013c). Variation in the nrDNA and 4 cp DNAs sequences were not completely correlated with species delimitation. Hybridization (past and present) and incomplete lineage sorting among the closely related taxa appear to present problems in DNA analysis.

Published on-line: www.phytologia.org *Phytologia* 95(2): 172-178 (May 1, 2013).

KEY WORDS: *Juniperus deppeana* varieties, *Cupressaceae*, DNA, nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, systematics, taxonomy.

The genus *Juniperus* is composed of approximately 75 species in three sections: *Caryocedrus* (1 species, Adams and Schwarzbach, 2012a), *Juniperus* (14 species, Adams and Schwarzbach, 2012a) and *Sabina* (approx. 60 species). Section *Sabina* is divided into three major clades (Mao et al., 2010, Adams 2011):

1. Serrate-leaf junipers of North America (21 species, Adams and Schwarzbach, 2011),
2. Turbinate-seed cones, single-seeded, entire-leaf junipers, eastern hemisphere (16 species, Adams and Schwarzbach, 2012b, 2013a, Zanon and Adams, 1976, 1979) and
3. Multi-seeded, entire-leaf junipers, both eastern and western hemispheres (23 species, Adams and Schwarzbach, 2012c, 2013b).

Recently, Adams and Schwarzbach (2006, 2013c) and Adams and Nguyen (2005) have reported on the taxonomy of *J. deppeana* and its varieties. The focus of the present study was to integrate the data from Adams and Schwarzbach (2011) and Adams and Schwarzbach (2013c) to give complete coverage of all the species, major varieties and formas (Adams 2011) of the serrate junipers North America using data obtained from extended sequencing of nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF.

MATERIALS AND METHODS

Specimens used in this study: *J. angosturana*, Adams 6881-6885, 21 km e of Cerritos, San Luis Potosi, MX, *J. arizonica*, Adams 7635-7638, Rock Hound State Park, NM, *J. ashei* var. *ashei*, Adams 10398, 10399, Bosque Blvd., Waco, TX, *J. ashei* var. *ovata*, Adams 7470, 7473, Ozona, TX, *J. californica*, Adams 10154, 10155, Hesperia, CA, *J. coahuilensis*, Adams 10241, 10242, km 18, n of Durango, MX, *J. comitana*, Adams 6858-6862, 14 km s Comitán, Chiapas, MX, *J. deppeana* var. *deppeana*, Adams 10539-10541, El Chico National Park, Hidalgo, MX; Adams 7632-7634, Sacramento Mtns., e of Alamogordo, NM, USA; Adams 10640-10642, Oak Creek Canyon-Flagstaff, AZ; *J. deppeana* var. *gamboana*, Adams 6863-6867, Comitán, Chiapas, MX; *J. deppeana* var. *patoniana*, Adams 6836-6839, km 152, w. of Durango (city), Durango, MX (P); *J. deppeana* var. *robusta*, Adams 10255-10256, w of La Ciudad, Durango, MX; *J. deppeana* f. *sperryi*, Adams 10626, Bridge Spring, Davis Mtns., TX, USA; Adams 11312, Munds Mtn., AZ; *J. deppeana* f. *zacatecensis*, Adams 6840-6842, 18 km w. Sombrette, Zacatecas, MX; *J. durangensis*, Adams 6832-6835, 52 km w El Salto, Dur., MX, *J. flaccida*, Adams 6893-6896, 22 km e San Roberto Jct., Nuevo Leon, MX, *J. grandis*, Adams 11964-11968, Meyers, CA, *J. jaliscana*, Adams 6846-6848, 19 km e Mex. 200 on road to Cuale, Jalisco, MX, *J. martinezii*, Adams 5950-5954, 42 km n Lagos de Moreno, Jalisco, MX, *J. monosperma*, Adams 10931-10934, Reserve, NM, *J. monticola* f. *monticola*, Adams 6874-6878, El Chico Natl. Park, Hidalgo, MX, *J. occidentalis*, Adams 8592-8596, Sisters, OR, *J. osteosperma*, Adams 6811-6815, Salt Lake City, UT, *J. pinchotii*, Adams 10463-10467, Meridian, TX, *J. poblana*, Adams 6868-6872, 62 km s Oaxaca, MX, *J. saltillensis*, Adams 6886-6890, 14 km e San Roberto Jct., Nuevo Leon, MX, *J. standleyi*, Adams 6852-6856, 24 km nw Huehuetango, Guatemala, *J. zanonii*, Adams 6898-6902, Cerro Potosi, Nuevo Leon, MX, *J. virginiana*, Adams 10231-10232, Knoxville, TN, USA. Voucher specimens are deposited at BAYLU herbarium Baylor University.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion. Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975, Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing the five gene regions (nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF) resulted in 4411 bp of data. A Bayesian tree based on these data (Fig. 1) shows the diversity in this section. *Juniperus californica* is the most distinct in its DNA. The varieties of *J. deppeana* are mostly unresolved reflecting their close relationship (Adams and Schwarzbach 2013c). The varieties of *J. ashei* (var. *ashei*, var. *ovata*) are in separate clades, supporting a taxonomic change (see below). *Juniperus arizonica*, treated as *J. coahuilensis* var. *arizonica* (see Adams 2004), is clearly very distinct in its DNA (at least in these 5 DNA regions sequenced) from *J. coahuilensis* (as well as *J. monosperma*, *J. pinchotii* and *J. angosturana*, Fig. 1). *Juniperus zanonii* has been treated as *J. monticola* f. *compacta*, but its DNA is very distinct and it is well supported in a clade with *J. saltillensis*, not with *J. monticola* (Fig. 1).

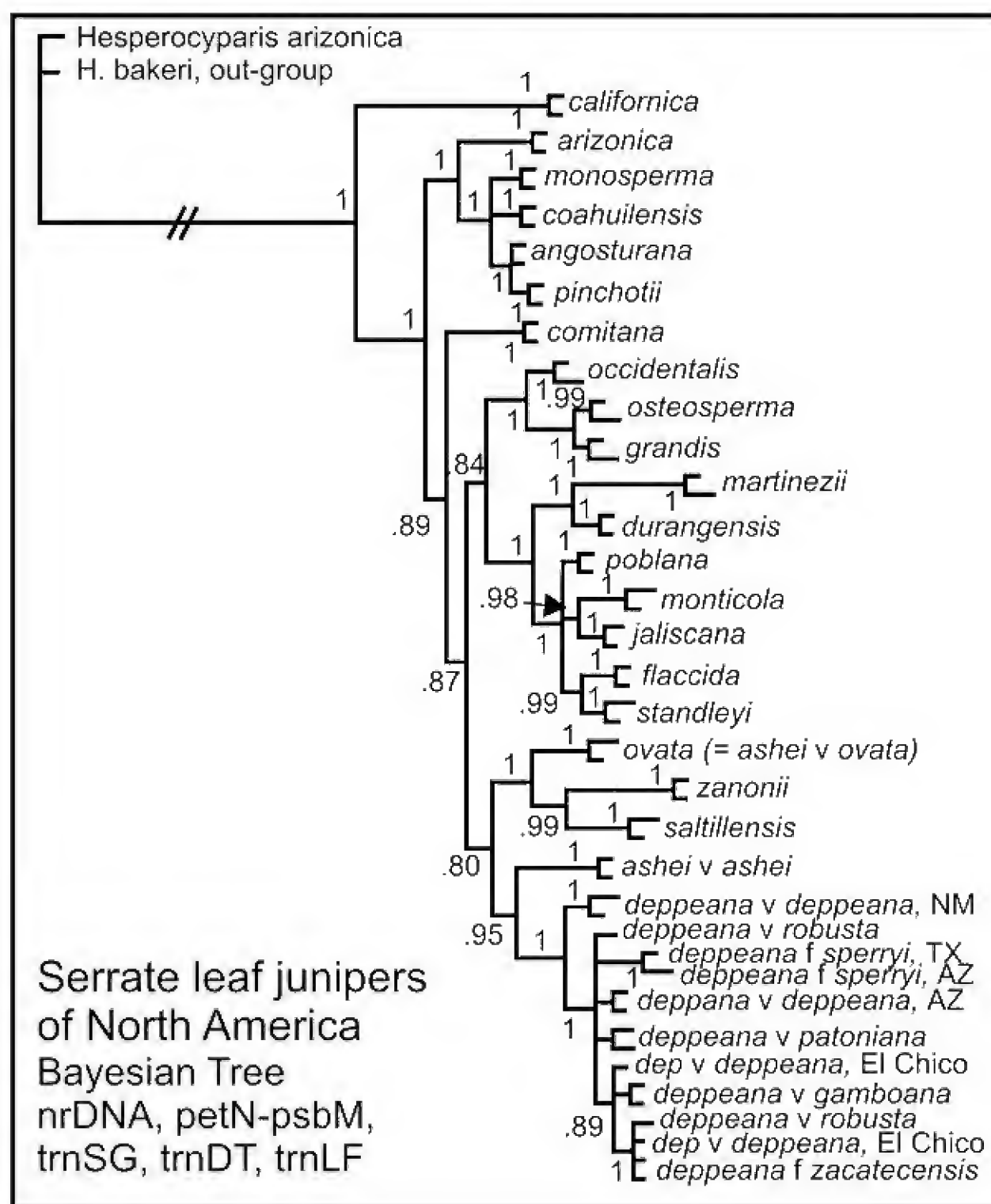


Figure 1. Bayesian tree of the serrate leaf junipers of North America. Numbers are posterior probabilities.

The Bayesian tree gives good information on the phylogeny within this group, but not about the magnitude of the mutational events (MEs) among the taxa. Analysis of the MEs (nucleotide substitutions plus indels) among these taxa (with *J. virginiana* as an outgroup) revealed multiple 191 MEs (found in more than one sample) and 21 single occurrence MEs. A minimum spanning network was constructed using the 191 MEs. Most of the taxa have accumulated many MEs. But three groups exhibit little variation: three western junipers; single seeded group; and *J. deppeana* group (Fig. 2).

Just as seen in the Bayesian tree (Fig. 1), *J. zanonii* is not near *J. monticola*, but its nearest neighbor is *J. saltillensis* (16 MEs, Fig. 2). In the single-seeded group, *J. arizonica* is the most distinct (8 MEs) and *J. angosturana* - *J. pinchotii*, the least distinct (1 ME, Fig. 2). Yet, *J. angosturana* and *J. pinchotii* are quite distinct in their morphology and leaf essential oils (Adams 2011). In the three western US junipers, *J. grandis* is separated by only 4 MEs from *J. osteosperma* and 7 MEs from *J. occidentalis* (Fig. 2). It seems evident that the nrDNA and 4 cp DNAs are not completely correlated with species delimitation.

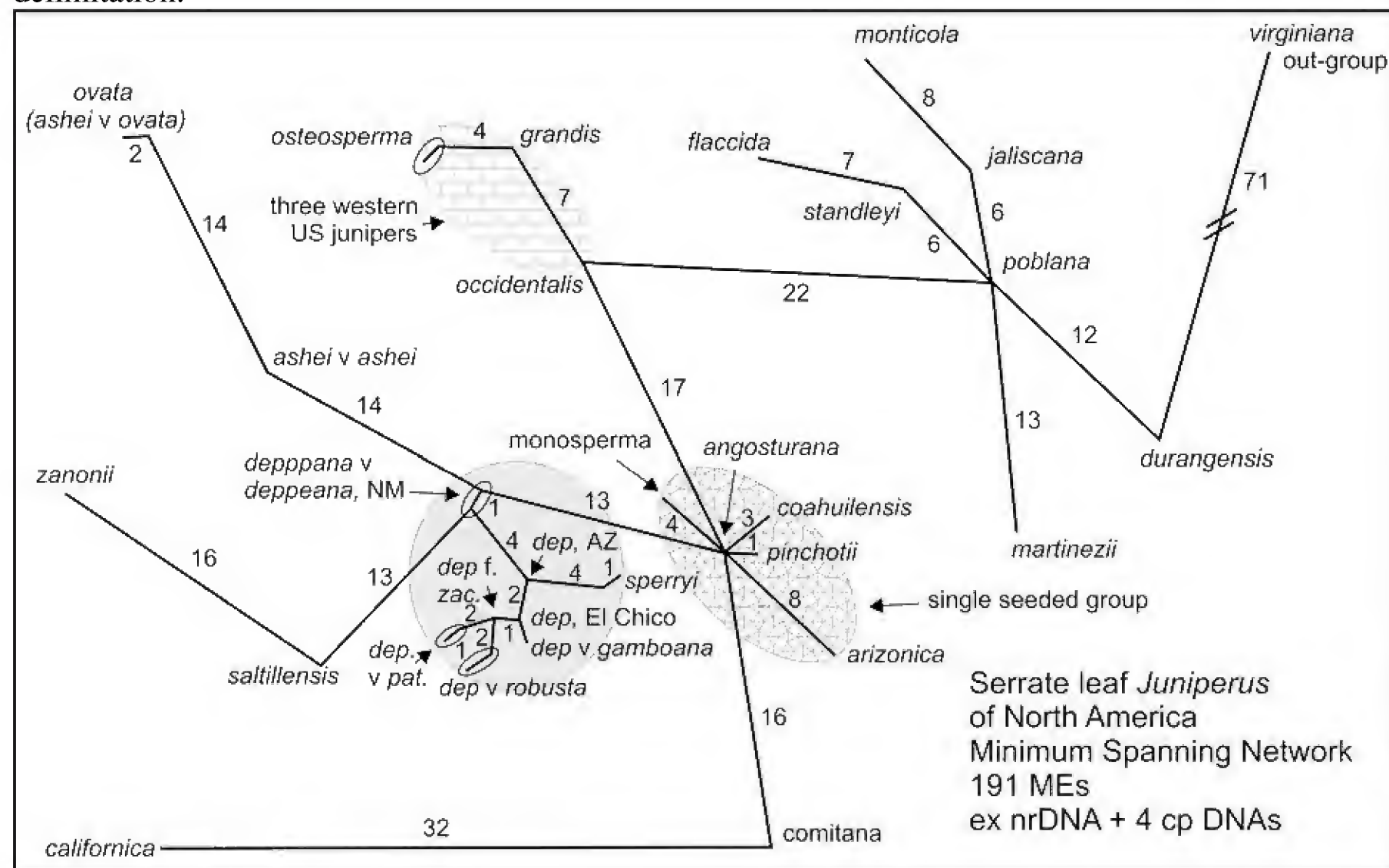


Figure 2. Minimum spanning network of serrate leaf *Juniperus* of North America. Numbers on the lines are the number of MEs (Mutational Events).

The varieties and forms of *J. deppeana* differ by 1 to 4 MEs and, on this basis, scarcely support varietal recognition. However, these taxa do differ in their morphology and leaf oils (Adams and Schwarzbach, 2013c; Zanoni and Adams, 1976, 1979).

Perhaps the most unusual taxa are *J. ashei* var. *ashei* and *J. a.* var. *ovata* that differ by 14 MEs (Fig. 2). These taxa overlap in their ranges near Ozona and New Braunfels, TX and have been shown to hybridize around New Braunfels (Adams, 2008). In view of the morphological and terpenoid differences (Adams 2011) and DNA differences, it seems appropriate to recognize *J. ashei* var. *ovata* as:

Juniperus ovata (R. P. Adams) R. P. Adams, *stat. & com. nov.*, oval gland juniper,

Basionym: *Juniperus ashei* Buch. var. *ovata* R. P. Adams, Phytologia 89(1): 17 (2007). TYPE: U. S. A., Texas, Crockett Co., 5 km w. Ozona, 6 Dec. 1994, R. P. Adams 7463 (holotype: BAYLU, Paratypes: R. P. ADAMS 7664, 7465, 7466, 7467 (BAYLU)).

A summary of the level of support for taxonomic taxa based on the present DNA sequence data is presented in table 1. The treatments of Adams (2011) and Farjon (2005, 2020) differ for *J. grandis*, *J. martiniezii*, *J. poblana* and *J. zanonii* (Table 1). In each of these cases, the DNA sequences in the present

study support the taxonomy of Adams (2011), except for *J. grandis*, in which specific status is only moderately supported. Notice that *J. grandis* and *J. osteosperma* differ by only 4 MEs (Table 1), but they are in strongly supported, separate clades (Fig. 1).

Table 1. Comparison of Adams and Farjon taxonomic treatments of taxa in this study. DNA sequencing support: ++ strong support; + support; +/- equivocal. NA = not analyzed, -- not mentioned. Nomenclatural changes are in boldface.

Adams(2011)	Farjon (2005, 2010)	Supported, this study
<i>J. angosturana</i> R. P. Adams	<i>J. angosturana</i>	+/- <i>J. angosturana</i>
<i>J. arizonica</i> (R. P. Adams) R. P. Adams	<i>J. arizonica</i>	++ <i>J. arizonica</i>
<i>J. ashei</i> Buchholz	<i>J. ashei</i>	++ <i>J. ashei</i>
var. <i>ovata</i> R. P. Adams	var. <i>ovata</i>	++ <i>J. ovata</i>
<i>J. californica</i> Carriere	<i>J. californica</i>	++ <i>J. californica</i>
<i>J. coahuilensis</i> (Martinez) Gaussen ex R. P. Adams	<i>J. coahuilensis</i>	+/- <i>J. coahuilensis</i>
<i>J. comitana</i> Martinez	<i>J. comitana</i>	++ <i>J. comitana</i>
<i>J. deppeana</i> Steudel var. <i>deppeana</i>	<i>J. d.</i> var. <i>deppeana</i>	+ <i>J. d.</i> var. <i>deppeana</i>
<i>J. deppeana</i> Steudel var. <i>deppeana</i>	<i>J. d.</i> var. <i>pachyphlaea</i>	+ <i>J. d.</i> var. <i>deppeana</i>
forma <i>elongata</i> R. P. Adams	--	NA
forma <i>sperryi</i> (Correll) R. P. Adams	var. <i>sperryi</i>	+ f. <i>sperryi</i>
forma <i>zacatacensis</i> (Mart.) R. P. Adams	var. <i>zacatacensis</i>	+/- f. or var. <i>zacatacensis</i> ?
var. <i>gamboana</i> (Mart.) R. P. Adams	<i>J. gamboana</i>	+/- var. <i>gamboana</i>
var. <i>patoniana</i> (Martinez) Zanoni	var. <i>robusta</i>	+/- f. or var. <i>patoniana</i> ?
var. <i>robusta</i> Martinez	var. <i>robusta</i>	+/- f. or var. <i>robusta</i> ?
<i>J. durangensis</i> Martinez	<i>J. durangensis</i>	++ <i>J. durangensis</i>
var. <i>topiensis</i> R. P. Adams & S. Gonzalez	--	NA
<i>J. flaccida</i> Schlecht.	<i>J. flaccida</i>	++ <i>J. flaccida</i>
<i>J. grandis</i> R. P. Adams	<i>J. occidentalis</i> var. <i>australis</i>	+ <i>J. grandis</i>
<i>J. jaliscana</i> Martinez	<i>J. jaliscana</i>	++ <i>J. jaliscana</i>
<i>J. martinezii</i> Perez de la Rosa	<i>J. flaccida</i> var. <i>martinezii</i>	++ <i>J. martinezii</i>
<i>J. monosperma</i> (Engelm.) Sarg.	<i>J. monosperma</i>	+ <i>J. monosperma</i>
<i>J. monticola</i> Martinez forma <i>monticola</i>	<i>J. monticola</i>	++ <i>J. monticola</i>
forma <i>compacta</i> Martinez	forma <i>compacta</i>	++ <i>J. zanonii</i> in part
forma <i>orizabensis</i> Martinez	forma <i>orizabensis</i>	NA
<i>J. occidentalis</i> Hook.	<i>J. occidentalis</i>	++ <i>J. occidentalis</i>
<i>J. occidentalis</i> f. <i>corbetii</i> R. P. Adams	--	NA
<i>J. osteosperma</i> (Torr.) Little	<i>J. osteosperma</i>	+ <i>J. osteosperma</i>
<i>J. pinchotii</i> Sudworth	<i>J. pinchotii</i>	+/- <i>J. pinchotii</i>
<i>J. poblana</i> (Martinez) R. P. Adams	<i>J. flaccida</i> var. <i>poblana</i>	++ <i>J. poblana</i>
<i>J. saltillensis</i> M. T. Hall	<i>J. saltillensis</i>	++ <i>J. saltillensis</i>
<i>J. standleyi</i> Steyermark	<i>J. standleyi</i>	++ <i>J. standleyi</i>
<i>J. zanonii</i> R. P. Adams	<i>J. monticola</i> f. <i>compacta</i>	++ <i>J. zanonii</i>

Farjon (2005, 2010) recognized *J. d. f. sperryi* as *J. d.* var. *sperryi* and the DNA data shows the two accessions of *sperryi* in a well-supported clade (Fig. 1). They differ by 4 MEs from *J. d.* var. *deppeana* (AZ, Fig. 2). However, the furrowed bark is so distinctive that it is easy to recognize *sperryi* among quadrangular bark trees, so the furrowed bark trees should be commonly reported. But, in fact, furrowed bark trees (*sperryi*) are very rarely reported (Adams and Schwarzbach, 2013c). *Juniperus d. f. sperryi* has yet to be found in a uniform population of trees with furrowed bark, but is found as one or a very few trees, interspersed with trees having quadrangular bark (*J. deppeana*). Every aspect of the scattered

occurrence of *sperryi* points to the presence of one or a few genes that is (are) expressed in *J. deppeana*. This pattern is typical of a *forma* not a variety.

As a group, the serrate-leaf junipers are closely related and found in deserts and semi-arid mountains of the southwestern United States and Mexico. It seems likely that many taxa are products of hybridization. Hybridization (past and present) and incomplete lineage sorting among the closely related taxa appear to present problems in the DNA analysis.

ACKNOWLEDGEMENTS

Tonnie Yanke for lab assistance. This research was supported in part with funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

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Phylogeny of *Juniperus* using nrDNA and four cpDNA regions.

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ABSTRACT

The Phylogeny of *Juniperus* is presented based on nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF sequencing (4411 bp) utilizing all currently recognized species. The major clades of the phylogenetic tree were congruent with the previously published phylogenetic tree of Mao et al. (2010) that had a subset of taxa of the current study. The lone species with serrate leaves in the eastern hemisphere, *J. phoenicea*, was found to be in a clade quite separated from the serrate junipers of North America. *Juniperus phoenicea* is referred to as 'pseudoserrate' to distinguish it from the semi-arid, serrate leaf junipers of the western hemisphere. Section *Sabina* is the most derived group and has radiated into niches in both the eastern and western hemispheres with approx. 60 species.

Published on-line: www.phytologia.org *Phytologia* 95(2): 179-187 (May 1, 2013).

KEY WORDS: *Juniperus*, phylogeny, *Cupressaceae*, DNA, nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT, trnL-trnF, evolution, migration.

The genus *Juniperus* is comprised of approx. 75 species in 3 sections:
sect. *Caryocedrus*, 1 species with large, blue, woody, 3-seeded cones, showing the fusing of 3 cone scales, with an Old World Mediterranean distribution (Adams, 2011, Adams and Schwarzbach, 2012a),

sect. *Juniperus*, 14 species, 12 only in the eastern hemisphere, one endemic to North America and one species, *J. communis*, being circumboreal, seed cones blue or red, often with 3 seeds (Adams and Schwarzbach, 2012a) and

sect. *Sabina* (approx. 60 species) with species about equally divided between the eastern and western hemispheres, seed cones with 1 to 13 seeds, blue, red-copper, rose, or brown (Adams, 2011).

Section *Sabina* is divided into three major clades (Mao et al., 2010, Adams 2011):

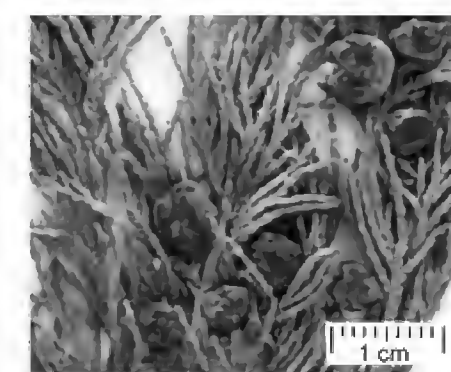
1. Serrate-leaf junipers of North America (21 species, Adams and Schwarzbach, 2011, 2013d),
2. Turbinate-seed cones, single-seeded, entire-leaf junipers, eastern hemisphere (16 species, Adams and Schwarzbach, 2012b, 2013a, Zanoni and Adams, 1976, 1979) and
3. Multi-seeded, entire-leaf junipers, both eastern and western hemispheres (23 species, Adams and Schwarzbach, 2012c, 2013b).



J. drupacea 1 cm



J. communis var. *communis* 1 cm



J. sabina 1 mm

The phylogenetic position of *Juniperus* (and Cupressaceae) in the plant kingdom (Fig. 1) shows *Juniperus* as a terminal clade and as one of the most advanced conifer genera (Rai et al., 2008). Mao et al. (2010) demonstrated that the closest relatives of *Juniperus* are *Cupressus* (of eastern hemisphere) and the *Hesperocyparis* - *Callitropsis* - *Xanthocyparis* clade (Fig. 2).

Although Mao et al. (2010) published a robust phylogeny of *Juniperus*, their principal purpose was to investigate the origins and evolutionary radiations of the major clades of *Juniperus*. As such, they utilized representative species from all clades, but not complete coverage of all known taxa. During the past few years, we have utilized DNA sequences to investigate the taxonomy of sections *Caryocedrus* and *Juniperus* (Adams and Schwarzbach, 2012a), section *Sabina*: serrate *Juniperus* of North America (Adams and Schwarzbach, 2006, 2012b, 2013a, 2013c, 2013d; Adams and Nguyen, 2005); the turbinate seed cones, eastern hemisphere (Adams and Schwarzbach, 2012b, 2013a) and the multi-seeded, entire-leaf junipers, both eastern and western hemispheres (Adams and Schwarzbach, 2012c, 2013b).

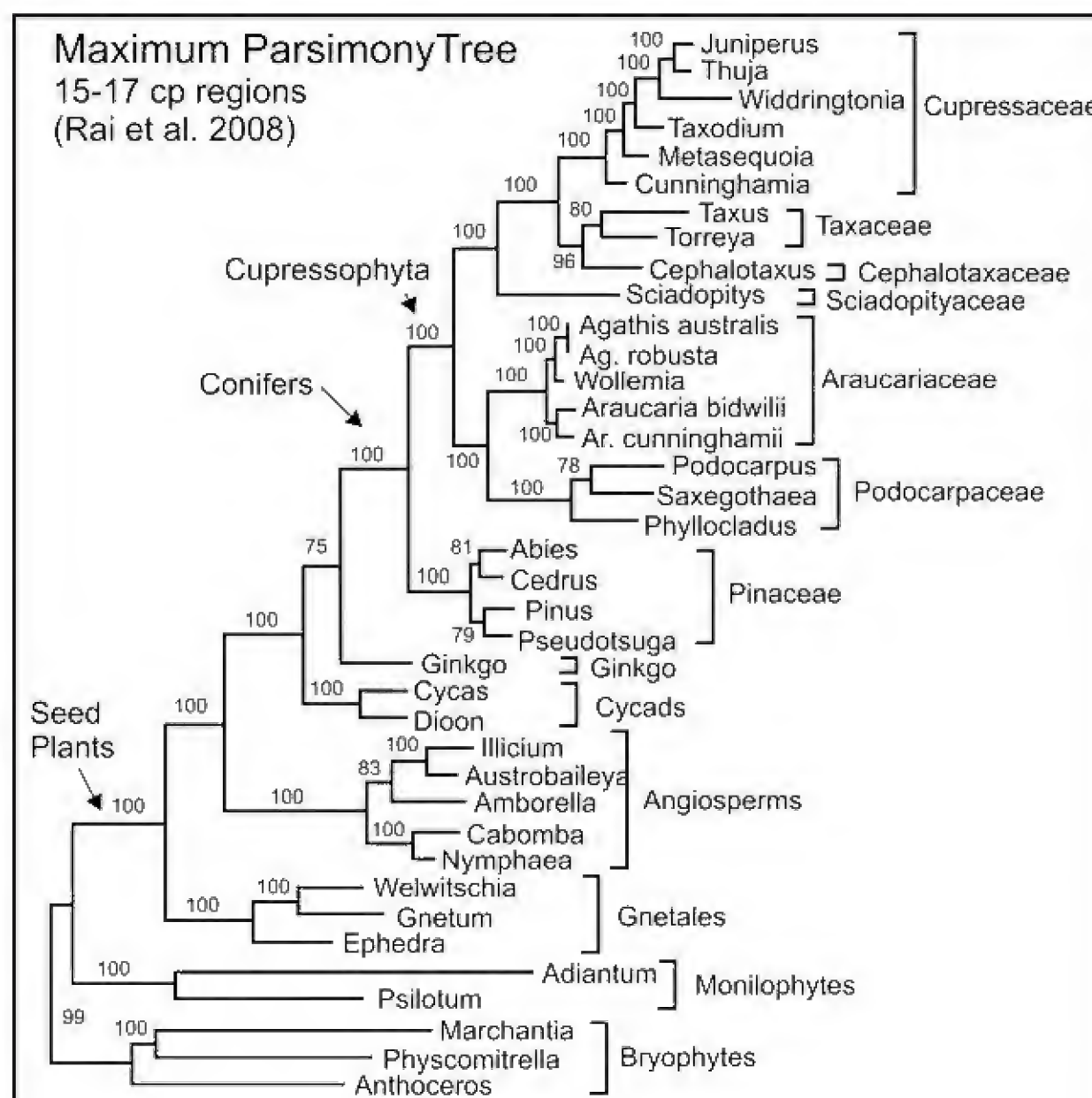


Figure 1. Maximum parsimony tree showing the position of *Juniperus*. Adapted from Rai et al. (2008).

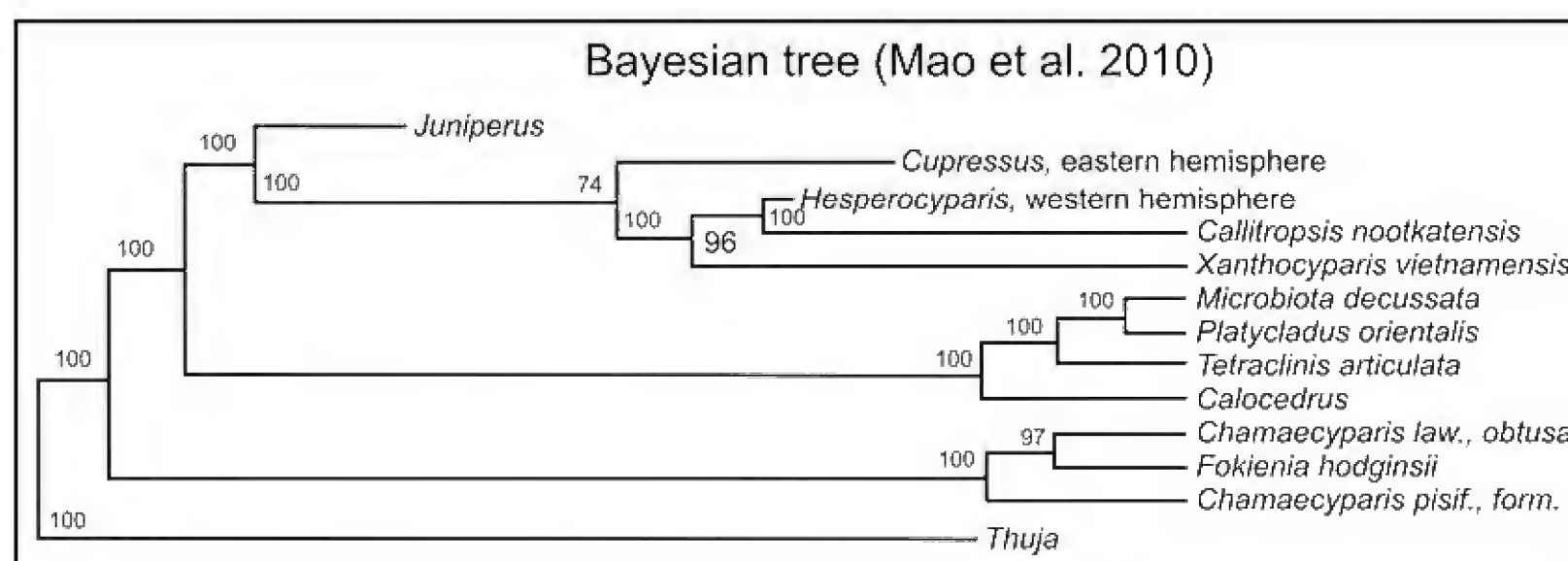


Figure 2. Simplified Bayesian tree with genera collapsed showing *Juniperus* relationship to *Cupressus* and *Hesperocyparis*. Adapted from Mao et al., 2010.

With the taxonomy and nomenclature having been addressed, the present report is to integrate these data into a robust phylogeny of *Juniperus* based on sequencing of nrDNA (ITS), petN-psbM, trnS-trnG, trnD-trnT and trnL-trnF including all known *Juniperus* species.

MATERIALS AND METHODS

Specimens used in this study: see Adams and Schwarzbach (2012a, 2012b, 2012c, 2013a, 2013b, 2013c). Voucher specimens are deposited at BAYLU herbarium Baylor University.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>), the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

RESULTS AND DISCUSSION

The phylogeny of *Juniperus* utilized the most informative gene regions (nrDNA, petN-psbM, trnSG, trnDT and trnLF). The tree is similar (Fig. 3) to Mao et al. (2010), except the positions of *J. californica*, *J. durangensis*, and for their clade IV which is now resolved into *excelsa* and *chinensis* groups (Fig. 3). The use of duplicate samples for most taxa appears to have stabilized the Bayesian tree in many places increasing branch support. In addition, all known taxa are included (approx. 100), compared with 51 taxa by Mao et al. (2010) and this has strengthened the tree.

Several 'problem' taxa present difficulties: *J. phoenicea* and *J. p. var. turbinata* stand loosely affiliated with sect. Sabina (Fig. 3). These taxa have small serrations on the leaf margins, but are denoted as 'pseudoserrate' (Fig. 3). It seems unlikely that serrate leaf margins in the eastern and western hemispheres is a homologous character, but has arisen independently as *J. phoenicea* is not in the clade with the serrate, semi-arid junipers of the western hemisphere. Also, *J. erectopatens* and *J. microsperma* form an unusual clade that does not nest into the *J. chinensis* clade (Fig. 3). *Juniperus ashei* and *J. a. var. ovata*, now *J. ovata* (R. P. Adams) R. P. Adams, are in separate clades (see Adams, 2008; Adams and Schwarzbach, 2013d for discussion).

Mao et al. (2010) used three *Juniperus* fossil dates: *J. pauli* (ca. ≥ 33.0 mya, cf. extant *J. sabina* and allies), *J. creedensis* (ca. ≥ 23.0 mya, cf. *J. californica* / *J. osteosperma*), and *J. desatoyana* (ca. ≥ 16.0 mya, cf. *J. occidentalis* / *J. osteosperma*). They postulated the serrate, semi-arid junipers migrated from the eastern to the western hemisphere via the North American Land Bridge (NALB) ca. 47 - 30.3 mya (Fig. 4).

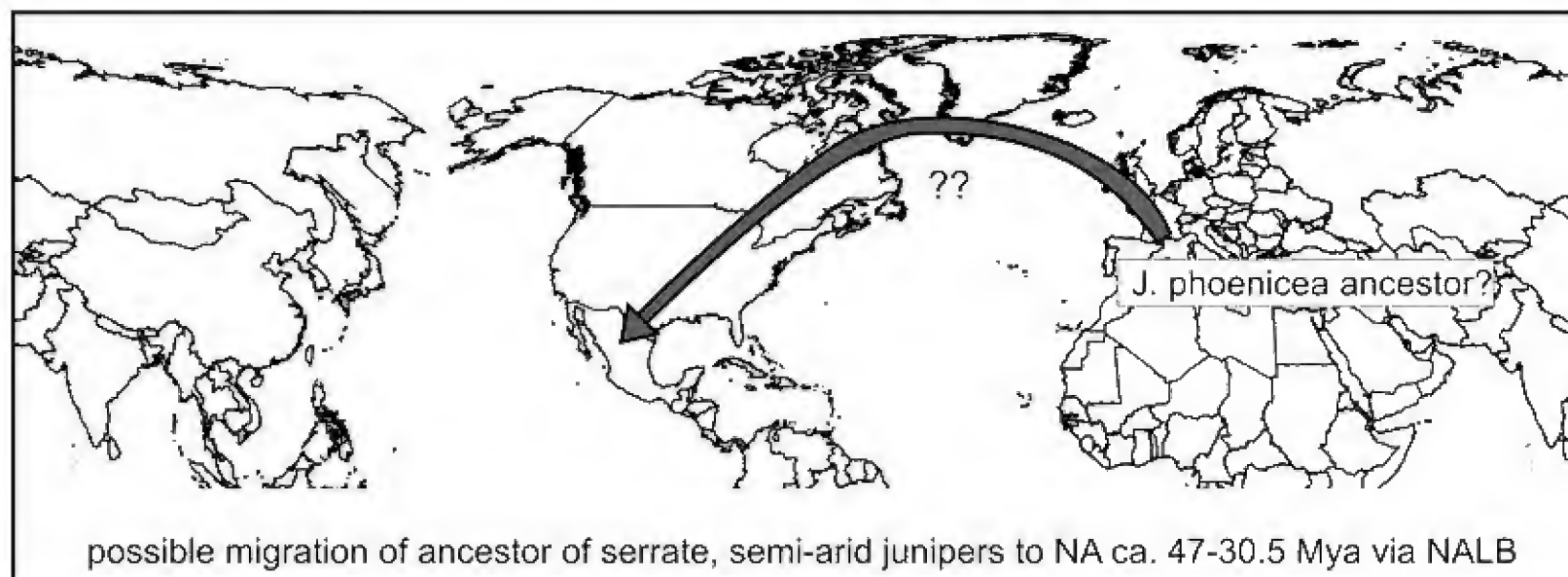


Figure 4. Possible migration of ancestor of the serrate, semi-arid junipers from the Mediterranean to the western hemisphere via the NALB.

The fossil *J. creedensis* of the Creede geoflora (ca. ≥ 23.0 mya) bears a striking resemblance to present-day *J. californica* (Fig. 5). Because the present-day *J. californica* appears little changed from the fossil, *J. creedensis*, it may be that the serrate junipers in North America are much older than thought. It might be noted that Axelrod (1987) described a second juniper from the Creede geoflora as *J. gracillensis* that he thought was similar to extant *J. flaccida*, but Wolfe and Schorn (1990) have identified the specimen as *Eleopoldia lipmanii* (Rosaceae).

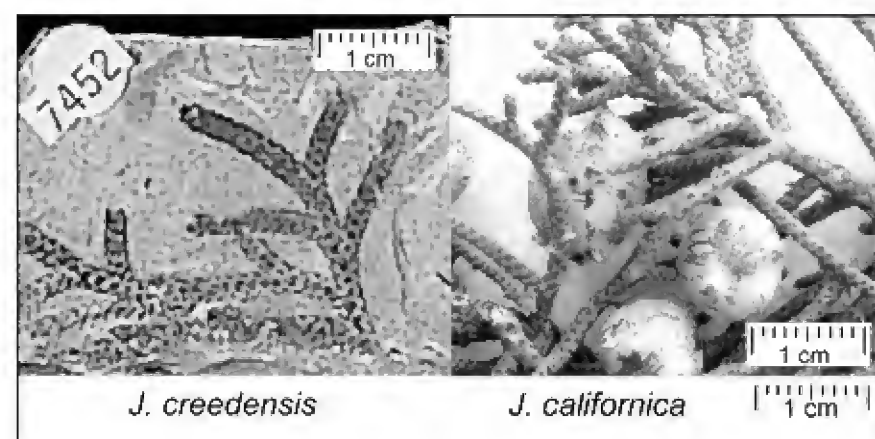


Fig. 5. *Juniperus creedensis* Axelrod paratype and present-day *J. californica*.

The Madrean-Tethyan vegetation belts in Eurasia and North America may have been continuous during the Eocene and Oligocene (Axelrod, 1975, Wen and Ickert-Bond, 2009), such that *Juniperus* section *Sabina* might have had a wider distribution (Fig. 6). So it is possible that the serrate-leaf junipers may have existed in the Madrean-Tethyan vegetation belts in both Eurasia and North America during the same period (Fig. 6), and there may have been exchanges via the North Atlantic Land Bridge (NALB). However, one should note the Madrean-Tethyan vegetation, depicted in Figure 6, predates the ages of any known juniper fossils.

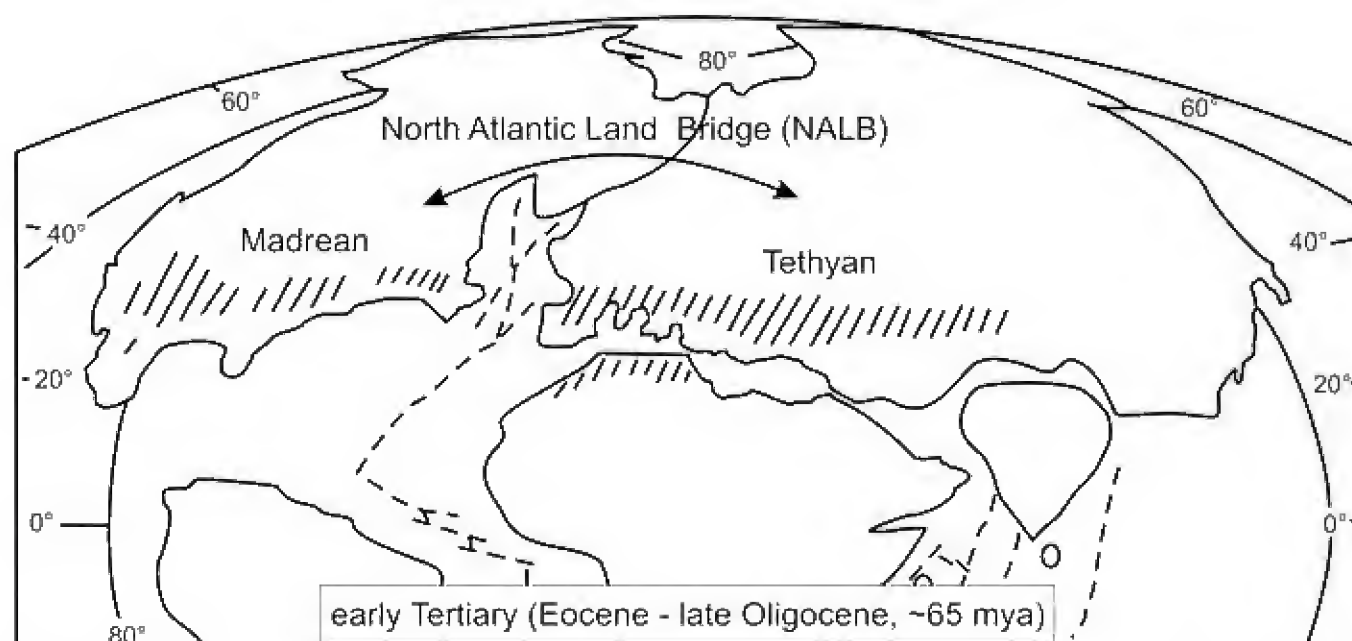


Figure 6. Madrean - Tethyan vegetation zones of Axelrod (adapted from Axelrod (1975) and Wen and Ickert-Bond (2009)).

It is unfortunate that the only serrate-leaf species (*J. phoenicea*) that is extant in the eastern hemisphere, has DNA so different that it is poorly grouped with any clade (Fig. 3). It is quite removed from the serrate junipers clade (Fig. 3). At present, it seems appropriate to consider *J. phoenicea* as 'pseudoserrate' and of a different lineage than the serrate junipers of North America. If the serrate leaves of *J. phoenicea* are not homologous to the serrate leaves of junipers in North America, then we are left with no extant (or known fossils) of truly serrate junipers in the eastern hemisphere.

The migration of the smooth leaf members of sect. *Sabina* to the western hemisphere is thought to be more recent (17.6-5.5 mya, Mao et al., 2010) and those dates are younger than the fossil *J. creedensis* of the Creede geoflora (Axelrod, 1987). Since *J. phoenicea* does not appear to be a true member of the serrate junipers and no serrate juniper fossils have been found in the eastern hemisphere, the serrate junipers may be endemic to the western hemisphere. Undoubtedly, additional fossils will be found some day to help resolve the question.

Mao et al. (2009) argues that the movement of sect. *Sabina* to the western (17.6-5.5 mya) is too young for migration across the North Atlantic Land Bridge (NALB), but possible via the Bering Land Bridge (BLB). Because sect. *Sabina* species such as *J. sabina* and *J. davurica* are quite cold adapted, they could have migrated to produce the ancestors that gave rise to the current, cold climate, western hemisphere species such as *J. horizontalis* and *J. scopulorum*. *Juniperus davurica* is the northeastern-most species in northeast Asia (in sect. *Sabina*) and could have provided ancestral stock to migrate across the Bering Land Bridge (Fig. 7). Notice that *J. davurica* - *J. sabina* are in a sister clade to the smooth-leaf juniper of North America (Fig. 3), supporting the concept of migration from northeastern Asia via the BLB (Fig. 7.)

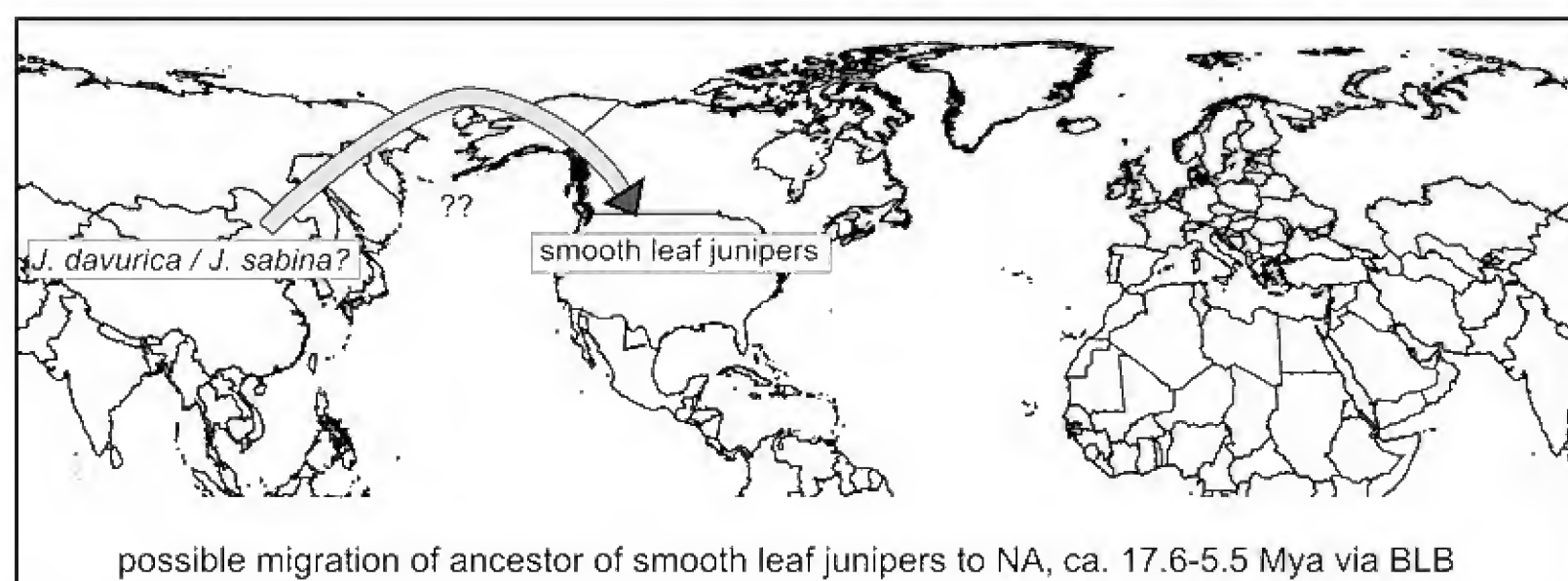


Figure 7. Possible migration pathway of the smooth leaf junipers of North America.

Juniperus communis is an interesting taxon in that it is the most weedy (invasive) species in sect. *Juniperus*. Its seed cones are especially juicy and attractive to birds. It is found in disturbed habitats as an invasive weed in Hungary and central Europe as well as in North America (as *J. c.* var. *depressa*). *Juniperus communis* and *J. c.* var. *depressa* form a boreal distribution in the higher latitudes around the northern hemispheres. Adams and Schwarzbach (2012a) examined the taxonomy of *J. communis* and found it to be very complex. The species is comprised of several morphological varieties that are closely linked by only a few mutations (Fig. 8). Notice the Kamchatka group (Fig. 8) is closely linked (6 mutational events, MEs) to *J. communis* var. *nipponica*, Japan, thence to *J. c.* var. *megistocarpa* (NA, 5 MEs).

The North America *communis* group is equally linked between the Japan and Europe-Central Asia groups. Thus, the linkage map gives equal support to the Bering Land Bridge and North Atlantic island hopping model for the origin of *J. communis* in North America. The situation was previously more

unclear when *J. jackii* was included in *J. communis* (*J. c.* var. *jackii*). However, *J. jackii* is clearly quite differentiated (20 MEs from *J. mairei*, Gansu, China; 21 MEs from *J. c.* var. *megistocarpa*, NA and Fig. 3), but the data is equivocal as to whether its origin is from the BLB or North Atlantic island hopping model. It grows on serpentine and volcanic basalt of quite recent origin in the Cascade Range of western Oregon/ northern California.

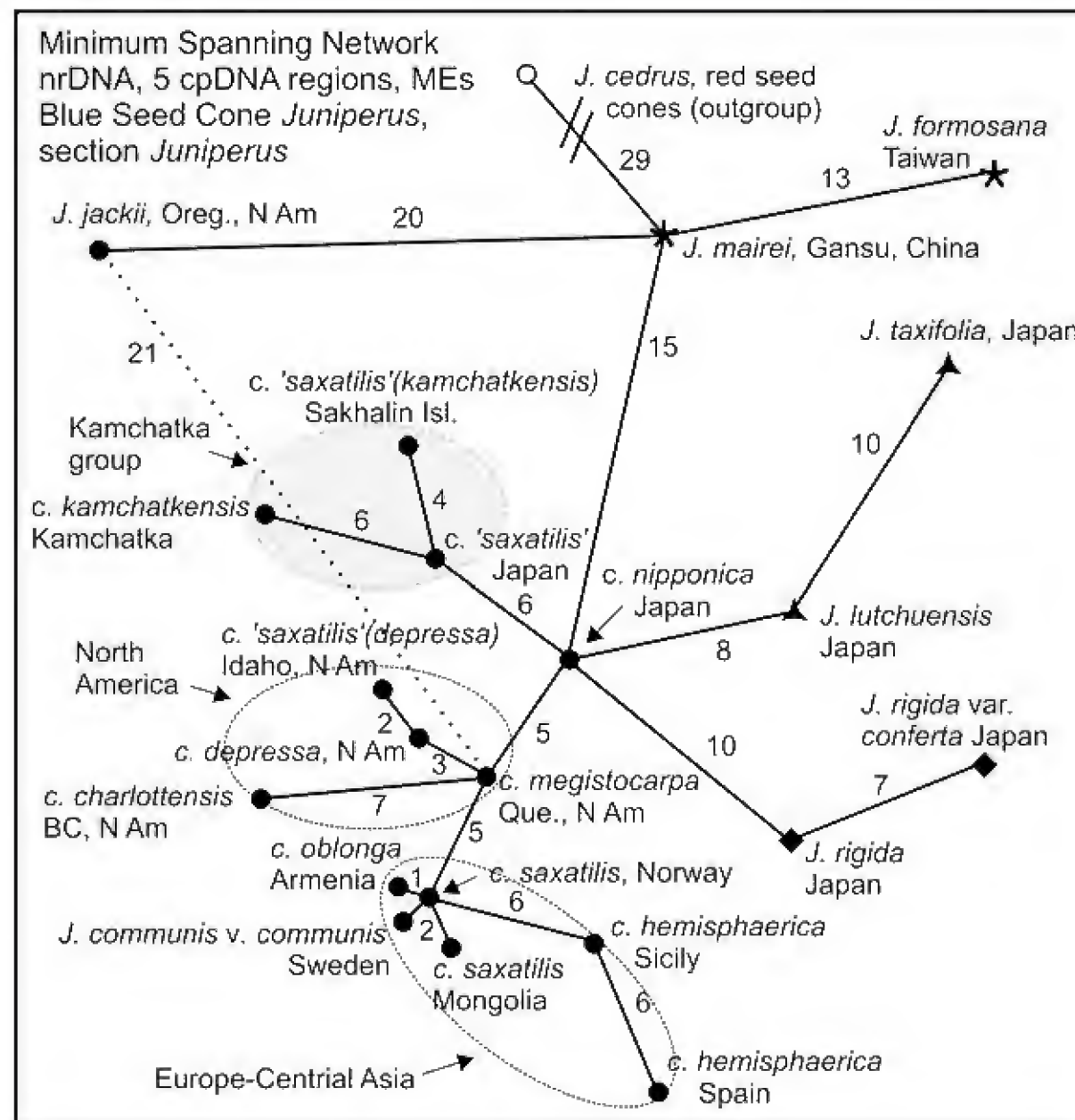


Figure 8. Minimum spanning network of blue seed cone junipers. Numbers on the links are MEs (mutational events). Adapted from Adams and Schwarzbach (2012a).

A diagrammatic representation of the possible migrations of *J. communis* (and *J. jackii*) is shown in Figure 9. The migration dates proposed by Mao et al. (2010) seem consistent with the recent habitat availability for *J. jackii* and support the observed lack of differentiation among morphological varieties of *J. communis* (Adams and Schwarzbach, 2012a).

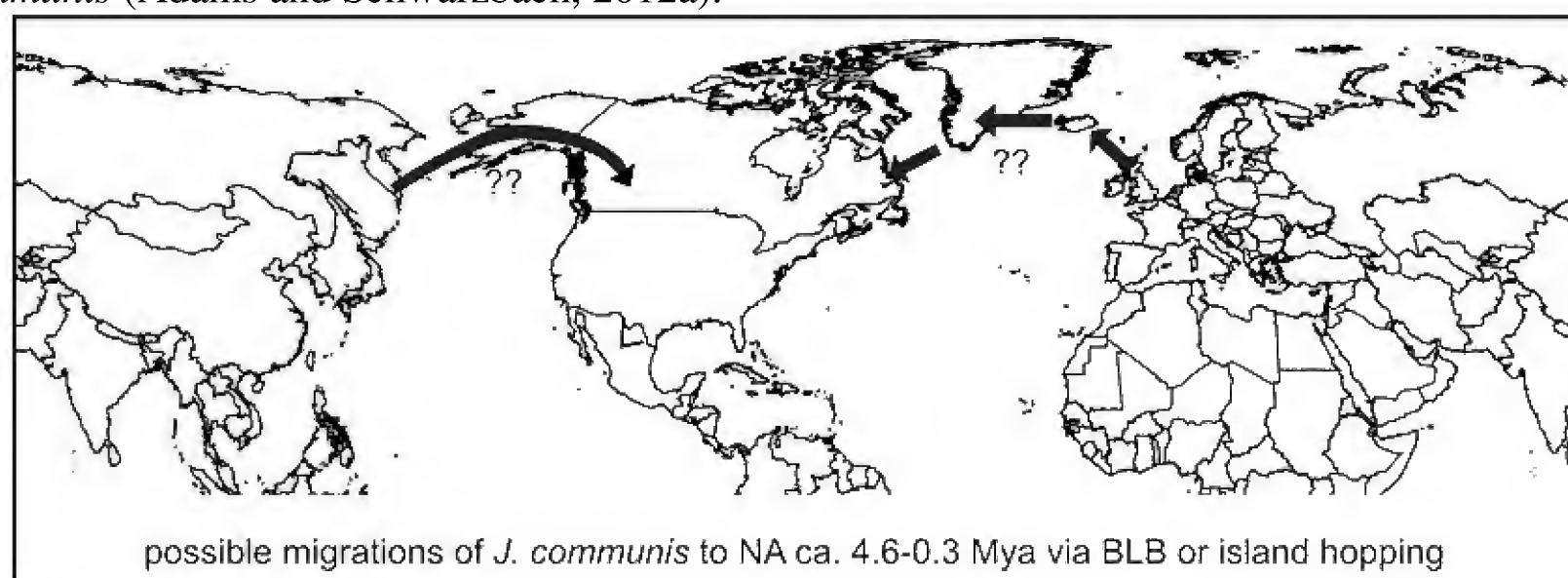


Figure 9. Possible migrations of *J. communis* to North America. Based on data in Mao et al. (2010).

Wen and Ickert-Bond (2009) summarized data from 17 studies concerning Madrean-Tethyan disjunctions. Their summaries are useful in the present discussion. They concluded (Fig. 10) that: 53% of the inter-continental migrations was by the North Atlantic Land Bridge; 40% was by long distance dispersal and 7% by the Bering Land Bridge (BLB). Their summary of the directional data indicated the origins as: 86% from eastern to western hemisphere; 7% from western to eastern hemisphere and for 7% the direction was uncertain (Fig. 10). This trend broadly supports the conclusions of Mao et al. (2010) and the present study.

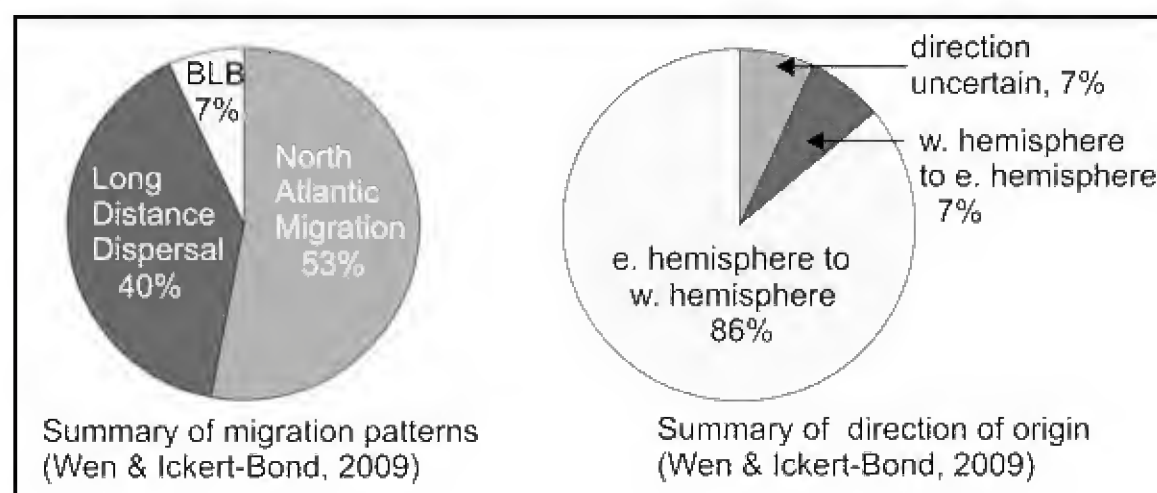


Figure 10. Summary of trends based on 17 studies of Madrean-Tethyan disjunctions. Adapted from Wen and Ickert-Bond (2009).

CONCLUSIONS

The present phylogenetic results are compatible with the results of Mao et al. (2010). In the present report, the clades are better defined and clade IV (Mao et al. 2010) is now resolved into three clades of the *J. excelsa* group and the *J. chinensis* group, with *J. erectopatens* and *J. microsperma* forming a somewhat intermediate clade. The lone species with serrate leaves in the eastern hemisphere, *J. phoenicea*, was found to be in a clade quite separated from the serrate junipers of North America. It appears that the evolution of serrate leaves occurred independently in the eastern hemisphere. *Juniperus phoenicea* is referred to as 'pseudoserrate' to distinguish it from the semi-arid, serrate leaf junipers of the western hemisphere. Section *Sabina* is the most advanced group and has radiated into niches in both the eastern and western hemispheres with approx. 60 species. Additional fossils are needed from older formations to clarify the evolution of the genus.

ACKNOWLEDGEMENTS

Thanks to the University of California Museum of Paleontology for the use of the photo of *Juniperus creedensis*, to Kangshan Mao and J-Q Liu for useful discussions, and Tonnie Yanke for lab assistance. This research was supported in part with funds from NSF grant DEB-316686 (A. Schwarzbach and R. P. Adams) and funds from Baylor University.

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Comparison of the volatile leaf and wood oils of the subspecies of *Pinus torreyana*: two isolated, narrow endemics in California

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ABSTRACT

The composition of the volatile leaf oils of *Pinus torreyana* subsp. *torreyana*, Del Mar, CA and subsp. *insularis* from Santa Rosa Island are reported. The leaf oil of subsp. *torreyana* is dominated by thunbergol (30.6%), iso-cembrene (25.5%), and cembrene (20.0%) with moderate amounts of limonene (6.9%) and trans- α -bergamotene (2.4%). In contrast, the leaf oil of subsp. *insularis* is dominated by a very large amount of thunbergol (67.1%), with moderate amounts of iso-cembrene (6.8%), cembrene (6.4%), limonene (3.6%) and β -phellandrene (3.6%). Trans- α -bergamotene and (E)- β -ocimene are unique to subsp. *torreyana*, whereas; β -phellandrene, trans-calamenene, 1-epi-cubenol, epi- α -muurolol and manoyl oxide are unique to subsp. *insularis*. The wood oils (turpentines) differed in the two subsp. (Zavarin et al., 1967), but shared very few compounds with the leaf oils. In short, either leaf or wood oils are sufficient to separate the subspecies. The differences in the oil compositions between the subspecies seems to indicate either a considerable period of isolation between the populations and/or genetic drift in the very small populations of subsp. *torreyana* and *insularis*. Published on-line www.phytologia.org *Phytologia* 95(2): 188- 191 (May 1, 2013).

KEY WORDS: *Pinus torreyana*, var. *insularis*, leaf oils, wood oils, alkanes, terpenes, diterpenes, evolution.

Pinus torreyana C. Parry ex Carr. (Torrey pine) is in subsection *Sabinianae* with *P. sabiniana* Doug. and *P. coulteri* D. Don. (Haller, 1986). *Pinus sabiniana* Doug. and *P. coulteri* are very widespread, but, in contrast, *P. torreyana* has one of the most limited ranges of any *Pinus* species (Haller, 1986). It has only two disjunct populations: in and around Del Mar, CA and 280 km northwest on Santa Rosa Island. Haller (1986) recognized the Santa Rosa elements as a new subspecies (*P. torreyana* subsp. *insularis* Haller). The new taxon differed in crown shape, needle color, cone width and shape, cone scale apex and seed width and color (Table 3, Haller, 1986). Haller noted that Zavarin et al. (1967) reported differences in the turpentine composition between plants from Del Mar and Santa Rosa Island.

The oleoresin oils (wood oils) of *Pinus* normally contain monoterpenes (turpentine) and non-volatile diterpene resin acids (rosin) (1996). However, the turpentine of *P. jeffreyi* Grev. & Balif. (Jeffrey pine) and *P. sabiniana* Dougl. (gray or digger pine) is composed of 95-99% n-heptane (with small amounts of undecane and other alkanes) and only less than a few percent monoterpenes (Mirov, 1948; Smith, 1967). Mirov (1961) reported 5% undecane in *P. torreyana* Perry and less than 0.1% heptane and 5% heptane in *P. coulteri* D. Don. Smith (1982) also reported varying amounts of heptane in the wood of *P. rudis* (0 - 32%) and *P. pseudostrobus* (0 - 47%). Smith (2000) published a massive recompilation of data from xylem monoterpenes in *Pinus*, but still reported that only the wood oils of *P. jeffreyi* and *P. sabiniana* contained high levels n-heptane (95-99%) in the monoterpene fraction.

Ekundayo (1988) reviewed the volatile constituents of *Pinus* needle oils for 33 species. He reported the compositions (from the literature) for mono- and sesquiterpenes, but not for di-terpenes. No data were presented for *Pinus torreyana*. Kurose et al. (2007) analyzed leaf oils from nine *Pinus* species, but not for *Pinus torreyana*.

Savage, Hamilton and Croteau (1996) appear to have been the first to compare the very volatile (monoterpenes and lower alkanes) of both wood and leaves of *P. jeffreyi*. They did not detect heptane in

the needle oil, but did find a progressive increase from the phloem (current 0.8, basal, 33.1%), to xylem (current, 35.4, sapwood, 80.6, heartwood, 95.2%).

Recently, Adams and Wright (2012) compared the compositions of the leaf and wood oils for *P. jeffreyi* and *P. sabiniana*. They reported that in each species the leaf oil compositions were almost completely different from the wood oils.

The purpose of this study is to report on the first analyses of the leaf oils from *P. torreyana* subsp. *torreyana* and subsp. *insularis* and to compare their leaf oils with published data for wood oils.

MATERIALS AND METHODS

Plant Specimens: *Pinus torreyana* subsp. *torreyana*, Adams 13652-13654, Del Mar (City), CA, *P. torreyana* subsp. *insularis*, Adams 13736-13737, Univ. of California Botanic Garden, Berkeley, CA, ex. Santa Rosa Island, e end of Torrey pine grove, Acc. 78.0073. Voucher specimens are deposited in the Herbarium, Baylor University (BAYLU).

Essential oil extraction: Fresh leaves were collected at 1.5 m above ground, on the south-facing side of each tree. Fresh leaves (200 g) were cut into 2 cm lengths to promote oil volatilization and steam distilled for 2 h using a circulatory Clevenger-type apparatus with a layer of diethyl ether as an oil trap (Adams, 1991). The oil samples were concentrated (diethyl ether removed) with nitrogen and the samples stored at -20°C until analyzed.

GC and GC/MS analysis: The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007, for operating details). The oils were run at both 60-246°C/3°C/min and at 40 °C, isothermal, 4 min, then 3°C/min to 246°C in order to resolve heptane and diethyl ether. Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds and the NIST database. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software without FID response factors.

RESULTS

The leaf oil of subsp. *torreyana* is dominated (Table 1) by thunbergol (30.6%), iso-cembrene (25.5%), and cembrene (20.0%) with moderate amounts of limonene (6.9%), and trans- α -bergamotene (2.4%). In contrast, the leaf oil of subsp. *insularis* is dominated (Table 1) by very large amount of thunbergol (67.1%), with moderate amounts of iso-cembrene (6.8%), cembrene (6.4%), limonene (3.6%), β -phellandrene (3.6%). Trans- α -bergamotene and (E)- β -ocimene were unique to subsp. *torreyana*, whereas β -phellandrene, trans-calamenene, 1-epi-cubenol, epi- α -muurolol and manoyl oxide were unique to subsp. *insularis*.

The wood oils (turpentine) differ between the subsp. (Zavarin et al., 1967), but share very few compounds with the leaf oils (Table 1). This is very similar to the situation for the compositions of the leaf and wood oils for *P. jeffreyi* and *P. sabiniana* (Adams and Wright, 2012), where these pines also share few components in the leaf and wood oils. It appears there is a different set of genes turned on in the leaves than in the wood for a few pine species (e. g., *P. jeffreyi*, *P. sabiniana*, and *P. torreyana*), whereas nearly all *Pinus* species have very similar leaf and wood oils (Smith, 2000). It is interesting that in the Cupressaceae (particularly *Juniperus*), all of the ca. 75 species (Adams and Schwarzbach, 2013) appear to have leaf and wood oils that share few terpenes (Adams, 1991). The role of chemical defenses

may be quite different between leaves and wood (Keeling and Bohlmann, 2006). This could account for the large differences between leaf and wood oil compositions.

Finally, it should be noted that data from either the leaf or wood oils are sufficient to separate subsp. *torreyana* from subsp. *insularis*. The differences in the oil compositions between the two taxa seem to indicate a considerable period of isolation between the populations, this resulting in genetic drift in the very small populations concerned.

ACKNOWLEDGEMENTS

Thanks to Holly Forbes, University of California Botanic Garden for providing samples of *P. torreyana* subsp. *insularis* and Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Table 1. Leaf and wood oil compositions (%) for *Pinus torreyana* subsp. *torreyana* from Del Mar, CA and subsp. *insularis* from Santa Rosa Island. Wood oil compositions are from Zavarin et al. (1967).

KI*	Compound	<i>torreyana</i> leaf oil	<i>insularis</i> leaf oil	<i>torreyana</i> wood oil	<i>insularis</i> wood oil
700	n-heptane	-	-	2.2	3.6
900	n-nonane	-	-	1.7	2.4
932	α -pinene	0.1	0.1	2.7	2.8
974	β -pinene	-	-	0.2	0.1
988	myrcene	0.3	0.3	2.9	1.7
1002	α -phellandrene	-	t	-	-
1020	p-cymene	-	0.2	-	-
1024	limonene	6.9	3.6	84.2	73.4
1025	β-phellandrene	-	3.6	0.1	8.7
1026	1,8-cineole	-	-	0.9	-
1032	(Z)- β -ocimene	t	t	-	-
1044	(E)-β-ocimene	1.9	-	-	-
1063	n-octanol	t	t	-	-
1086	terpinolene	t	-	-	-
1095	linalool	0.1	0.1	-	-
1100	undecane	-	-	6.1	7.4
1100	nonanal	0.1	-	-	-
1118	cis-p-menth-2-en-1-ol	-	t	-	-
1136	trans-p-menth-2-en-1-ol	-	t	-	-
1141	camphor	t	t	-	-
1148	citronellal	-	t	-	-
1174	terpinen-4-ol	t	0.2	-	-
1186	α -terpineol	t	t	-	-
1201	decanal	-	-	0.4	-
1223	citronellol	t	0.2	-	-
1260	2-decenal	-	t	-	-
1284	bornyl acetate	t	0.9	-	-
1298	carvacrol	-	0.1	-	-
1322	methyl geranate	-	0.3	-	-
1400	tetradecane	-	-	t	-
1407	longifolene	-	-	0.4	2.8
1417	(E)-caryophyllene	1.2	1.0	-	-
1432	trans-α-bergamotene	2.4	-	-	-
1452	α -humulene	0.2	0.1	-	-
1454	(E)- β -farnesene	0.2	0.1	-	-
1484	germacrene D	-	0.1	-	-
1491	(E)-methyl isoeugenol	0.1	-	-	-
1505	(E,E)-α-farnesene	0.2	t	-	-
1521	trans-calamenene	-	0.2	-	-
1561	E-nerolidol	1.5	0.7	-	-
1574	germacrene-D-4-ol	-	t	-	-
1627	1-epi-cubenol	-	0.2	-	-
1638	epi- α -muurolol	-	0.3	-	-
1722	(2Z,6E)-farnesol	1.5	1.8	-	-
1814	hexadecanal	1.3	0.2	-	-
1937	cembrene	20.0	6.4	-	-
1943¹²	iso-cembrene	25.2	6.8	-	-
1965	(3Z)-cembrene A	1.0	0.7	-	-
1987	manoyl oxide	-	0.4	-	-
2048	thunbergol (isocembrol)	30.6	67.1	-	-

KI* = Kovats Index (linear) on DB-5 column from Adams (2007). Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

***Acacia* s.l. Dominated Thorn-scrub Woodland Communities at Harris Ranch,
Uvalde County, Texas.**

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ABSTRACT

Woody species have dramatically increased in density and cover in the semiarid grasslands in southwestern North America during the past 150 years. This brush encroachment involves mostly native species, not uncommonly the dominants being woody members of the Fabaceae. Anthropomorphic factors are mostly responsible, particularly intense grazing pressure from cattle and sheep and fire suppression. At Harris Ranch, Uvalde County, Texas, thorn-scrub woodlands are common. Dominated by thorny woody legume species, the most common taxa are *Senegalia berlandieri* [= *Acacia berlandieri* (guajillo, fern acacia)] and *Senegalia wrightii* [= *Acacia wrightii* (Wright's senegalia)]. In the two communities studied, these two legume species dominated with importance values between 58 and 159 (possible 200), with 13 to 18 woody and succulent species present in the community. Published on-line www.phytologia.org *Phytologia* 95(2): 192-201 (May 1, 2013).

KEY WORDS: community structure, *Senegalia berlandieri*, *S. wrightii*, thorn-scrub woodland.

Much of the South Texas Plains and the Edwards Plateau once supported a grassland or open savanna with a ground layer of short grasses and forbs. The woody legume *Prosopis glandulosa* (honey mesquite) along with lesser numbers of other shrubs and trees were usually clustered or scattered throughout this grassland (Van Auken 2000). During the past 200 years the composition and structure of these semiarid grasslands has changed, mostly the result of dramatic increases in native woody species (Johnston 1963, Archer et al. 1988, Archer 1989). Most of this change to brushy thorn-scrub woodland is apparently due to anthropogenic forces, primarily overgrazing by domestic livestock and fire suppression (Correll and Johnston 1970, Archer et al. 1988, Ruthven et al. 2000, Van Auken 2000, Ruthven 2001). These well-armed species are common throughout the arid and semi-arid environments of the South Texas Plains and the Edwards Plateau (Isely 1998), and along with *Prosopis glandulosa* are important sources of animal fodder, fuel, and timber (Fagg and Stewart 1994).

Thorn-scrub vegetation is common on much of Harris Ranch, which is located in the southern foothills of the Edwards Plateau in the northern part of Uvalde County, Texas. Two distinct thorn-scrub communities were encountered, a lowland community dominated by *Senegalia wrightii*/*Ziziphus obtusifolia*, and an upland community dominated by *Senegalia berlandieri*/*Opuntia engelmannii*/*Vachellia rigidula*. The objective of this study was to examine the structure and composition of these two thorn-scrub woodland communities on the Harris Ranch to understand better the importance, distribution, and habitat preferences of thorny legume species.

METHODS

Study Area: Harris Ranch is located near Cline, 20 miles west of Uvalde, Uvalde County, Texas at the northern edge of the South Texas Plains ecological region in the foothills of the Edwards Plateau. Managed by the Texas A & M University, Agricultural Research and Extension Center, Uvalde, the ranch is not deer-proof fenced, about 6,764 ha in size, and utilizes a cattle stocking rate of one animal unit to 35 ha (Cooper et al. 2008). Hot summers and mild winters characterize the climate of Uvalde, Texas. In a typical year the temperature varies from 4°C (40°F) to 36°C (97°F), and is rarely below -1°C (31°F) with a growing season of 250 to 365 days. The mean annual precipitation (96 year record) is 61.7 cm distributed bimodally with peaks occurring in late spring (May-June), and early fall (September-October). Short-term periods of drought are common and rainfall can be highly variable with annual extremes from 23.6 cm to 114.4 cm (Owens et al. 2002). Topography is gently rolling with the average elevation about 350 m above mean sea level.

Survey Procedures: During the summer of 2003 the two thorn-scrub woodland communities were studied. Both sites were nearly level areas with minimal disturbance other than grazing. At each site a line transect was randomly established near the center of the long axis of each community. At 30 m intervals along the length of the transect, circular plots 0.03 ha in size were located (10 plots) and all woody plants and succulents greater than 0.4 m tall were identified and their height and average crown diameter determined to the nearest dm. Data from the plots were used to determine density, average cover, relative density, relative cover, and importance value (IV) for each species at each site. The IV is calculated as the sum of the relative density and relative cover (Seigler et al. 2007).

Floristic Composition: Harris Ranch was visited five times during the growing seasons of 2002 to 2005. During these visits voucher specimens of all vascular plant species observed in and around each of the study sites, were collected, identified, and deposited in the herbarium of the University of Illinois, Urbana, Illinois (ILL). As both sites were heavily grazed most collections were from habitat not accessible to cattle. Nomenclature follows Jones et al. (1997), exotic species follows Nesom (2010).

RESULTS

At both study sites, *Senegalia* species dominated and accounted for at least 25% of the total importance value (total possible IV of 200). The upland community, on the thin soils of calcareous ridges and caliche cuestras, was dominated by *Senegalia berlandieri* (guajillo), *Opuntia engelmannii* (prickly pear), and *Vachellia rigidula* (blach bush). These three species accounted for more than 90% of the total IV, the remaining 10 species being uncommon (Table 1). The lowland community, on the relatively deep alluvial soil of a shallow valley with a small stream, was dominated by *Senegalia wrightii* (Wright's senegalia) and *Ziziphus obtusifolia* (lotebush, gumdrop tree). These two species, with IV's of 57.9 and 31.4 respectively, accounted from about 45% of the total IV. In this community, four other species (*Condalia hookeri*, *Quercus virginiana*, *Colubrina texensis*, *Prosopis glandulosa*) had IV's greater than 10, the remaining 11 species being uncommon (Table 2)

The number of woody and succulent species recorded for the two thorn-scrub woodland sites ranged from 13 to 18 with a total of only 21 different species recorded. Of the 21 species encountered, 10 occurred on both sites: *Aloysia gratissima* (white bush), *Berberis trifoliata* (agarito), *Condalia hookeri* (brazil), *Condalia spathulata* (squaw-bush), *Diospyros texana* (Texas persimmon), *Forestiera angustifolia* (narrowleaf forestiera), *Leucophyllum frutescens* (purple sage), *Opuntia engelmannii* (prickly pear), *O. leptocaulis* (tasajillo), and *Schaefferia cuneifolia* (desert yaupon). *Proposis glandulosa*, a common species that usually dominates thorn-scrub woodlands, was only encountered in the lowland thorn-scrub community, but was common on parts of Harris Ranch.

Diversity was relatively low with only 121 species of vascular plants encountered (Appendix I). No fern, “fern-allies” or gymnosperms were collected. Of the taxa encountered, 23 were monocots in four families, and 98 were dicots in 37 families. Non-native (exotic) species accounted for five taxa, about 4% of the species collected. As is typical of prairie and thorn-scrub vegetation, Poaceae was the most common family with 19 species, Asteraceae was second with 17 species. No state endangered or threatened species were encountered.

DISCUSSION

The thorn-scrub vegetation of Harris Ranch and surrounding area was representative of that associated with the foothills and much of the uplands of the Edwards Plateau and the adjacent South Texas Plains (Correll and Johnston 1970, Van Auken 2000, Owens et al. 2002). In much of this rangeland, *Prosopis glandulosa* was the dominant species, with about 10 to 15 other woody or succulent, mostly armed species, varying in abundance and composition. This woodland community, where dominant trees were 3 m tall and formed a 26-60% canopy, would be equivalent to the Deciduous Woodland, Mesquite-Huisache Series (*Prosopis glandulosa*-*Vachellia farnesiana*) of Diamond et al. (1987) with other thorny legume species replacing huisache (*V. farnesiana*). Also referred to as the mesquite/mixed acacia savanna, this community varies extensively in overstory composition with *Prosopis glandulosa* not always present or only in low concentrations.

In south central Texas the dominant woody species of these thorn-scrub communities is mostly *Prosopis glandulosa*, but various species of the genera *Senegalia* and *Vachellia* are also common, and may dominate, sometime with a total lack of *Prosopis glandulosa*. The common species of these two genera in Texas include *Senegalia berlandieri* [= *Acacia berlandieri* (guajillo, fern acacia)], *S. greggii* [= *Acacia greggii* (catclaw acacia)], *Vachellia bravoensis* [= *Acacia schaffneri* var. *bravoensis* (huisachillo)], *V. farnesiana* [= *Acacia farnesiana* (huisache)], and *V. rigidula* [= *Acacia rigidula* (blackbrush)] (Seigler et al. 2007, 2011). At Harris Ranch, *Prosopis glandulosa* was not the dominant woody species, being replaced by other woody legumes.

Senegalia berlandieri, along with lesser amounts of *Vachellia rigidula*, dominated limestone ridges and caliche cuevas of the Harris Ranch (Table 1). This community, in which the dominants were shrubs or small trees 0.5 to 4 m tall, and formed 26 percent or more of the total canopy, would be equivalent to the Deciduous Shrubland, Blackbrush Series (*Vachellia rigidula*) of Diamond et al. (1987). *Vachellia rigidula* appeared to be fairly site specific at the Harris Ranch being restricted to the calcareous ridges. This species appears to be well adapted to dry sites with high levels of available calcium. *Senegalia berlandieri*, in contrast, was a component of disturbed habitats, often along roadsides and in arroyos, but also an important stand component on the limestone ridges. Common throughout southern and western Texas, this species is abundant on limestone ridges and caliche cuevas (Correll and Johnston 1970, Isely 1998, Seigler et al. 2007, 2011).

Senegalia wrightii was the dominant species in the thorn-scrub woodland community on alluvial soil at Harris Ranch (Table 2). This taxon is closely related to *S. greggii* and has, in the past, been considered a variety of *S. greggii* (Isely 1969). *Senegalia berlandieri* and *S. wrightii* occasionally hybridize, producing the uncommon *S. x turnerii*. A population of this hybrid was observed at Harris Ranch on a hillside near the *S. wrightii* thorn-scrub community. On this hillside, both parental species were encountered along with numerous individuals of the resulting hybrid (Seigler et al. 2012).

A species of calcareous soils, *Senegalia wrightii* is known from near sea level to about 800 m in southern Arizona and southwestern Texas in the United States, and the states of Baja California Sur, Chihuahua, Coahuila, Nuevo Leon, and Tamaulipas, Mexico. Until this study, we have never seen an extensive population of *S. wrightii*, generally finding one, or a few individuals along roadsides, in

pastures, or other disturbed areas. In this thorn-scrub community many individuals exceed 3 m in height: *S. wrightii*, *Quercus virginiana*, and *Celtis laevigata*. The remaining species were mostly less than 2 m tall. *Prosopis glandulosa*, sixth in IV, was mostly present as small individuals rarely exceeding 2 m tall.

Though some of the acacia species at Harris Ranch have distinct habitat preferences, the reason for their continued importance and the continued prevalence of thorn-scrub woodland communities they dominate is not entirely clear. Most information suggests that overgrazing and fire suppression were the primary causes of this encroachment (Lehmann 1969, Van Auken 2000). When much of the South Texas Plains and the Edwards Plateau was covered with open savanna containing a dense groundcover of grasses and forbs, wildfires were frequent and of sufficient intensity to prevent encroachment by native woody species. However, overgrazing by livestock reduced the fuel load. At the same time, fire suppression allowed for a significant decrease in fire frequency creating ideal conditions for the rapid expansion of native invaders. Presently, prescribed burns are being used as one way to reduce shrub and tree densities and increase the extent of prairie (Owens et al. 2002). These fires many not be of sufficient intensity and frequency to reduce the extent of thorn-scrub woodlands significantly.

ACKNOWLEDGEMENTS

We wish to acknowledge support of this work by a grant from the International Arid Lands Consortium (O1R005), a grant from the National Science Foundation (NSF DEB 04-15803), and to thank the Texas Parks and Wildlife Department, and in particular D. C. Ruthven, J. F. Gallagher, and D. R. Synatzske of the Chaparral Wildlife Management Area, and M. K. Owens with the Texas Agricultural Experiment Station for their assistance with the project. We also appreciate the critical review of the manuscript by Dr. David Riskind, Texas Parks and Wildlife Department, Austin, and William McClain, Adjunct Research Associate in Botany, Illinois State Museum, Springfield, Illinois.

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Table 1. Density (#/ha) by height class (m), average crown cover (m²), relative frequency, relative crown cover, and importance value (I.V.) of the species encountered at the *Senegalia berlandieri*/*Vachellia rigidula* site at Harris Ranch, Uvalde County, Texas.

Species	Density (#/ha) by height class (m)					Av. Cover	Rel. Den.	Rel. Cov.	I.V.
	0.4-1	1-2	2-3	3+	Totals				
<i>Senegalia berlandieri</i>	1112	217	20	--	1349	2.657	74.8	84.5	159.3
<i>Opuntia engelmannii</i>	230	--	--	--	230	1.643	12.7	8.9	21.6
<i>Vachellia rigidula</i>	27	10	10	--	47	3.080	2.6	3.4	6.0
<i>Leucophyllum frutescens</i>	43	--	--	--	43	0.739	2.4	0.8	3.2
<i>Berberis trifoliata</i>	37	--	--	--	37	0.353	2.0	0.3	2.3
<i>Diospyros texana</i>	7	13	--	--	20	2.055	1.1	1.0	2.1
<i>Opuntia leptocaulis</i>	33	--	--	--	33	0.441	1.8	0.3	2.1
<i>Condalia hookeri</i>	13	--	--	--	13	1.174	0.7	0.4	1.1
<i>Aloysia gratissima</i>	10	3	--	--	13	0.577	0.7	0.2	0.9
<i>Eysenhardtia texana</i>	7	--	--	--	7	1.324	0.4	0.2	0.6
<i>Forestiera angustifolia</i>	7	--	--	--	7	0.161	0.4	0.0	0.4
<i>Condalis spathulata</i>	3	--	--	--	3	0.385	0.2	0.0	0.2
<i>Schaefferia cuneifolia</i>	3	--	--	--	3	0.196	0.2	0.0	0.2
Totals	1532	243	30	--	1805		100.0	100.0	200.0

Table 2. Density (#/ha) by height class (m), average crown cover (m²), relative frequency, relative crown cover, and importance value (I.V.) of the species encountered at the *Senegalia wrightii* site at Harris Ranch, Uvalde County, Texas.

Species	Density (#/ha) by height class (m)					Av. Cover	Rel. Den.	Rel. Cov.	I.V.
	0.4-1	1-2	2-3	3+	Totals				
<i>Senegalia wrightii</i>	57	17	23	50	147	6.796	12.6	45.3	57.9
<i>Ziziphus obtusifolia</i>	213	40	3	--	256	0.795	22.1	9.3	31.4
<i>Condalis hookeri</i>	127	27	--	--	154	0.887	13.2	6.2	19.4
<i>Quercus virginiana</i>	--	--	--	10	10	34.780	0.9	15.8	16.7
<i>Colubrina texensis</i>	90	23	--	--	113	1.109	9.7	5.7	15.4
<i>Prosopis glandulosa</i>	133	3	3	--	139	0.173	12.0	1.1	13.1
<i>Opuntia engelmannii</i>	70	3	--	--	73	0.965	6.3	3.2	9.5
<i>Aloysia gratissima</i>	20	30	10	--	60	1.181	5.2	3.2	8.4
<i>Diospyros texana</i>	27	13	7	--	47	0.906	4.0	1.9	5.9
<i>Celtis laevigata</i>	30	13	--	3	46	0.563	4.0	1.2	5.2
<i>Opuntia leptocaulis</i>	37	3	--	--	40	0.826	3.4	1.5	4.9
<i>Guajacum angustifolium</i>	17	7	--	--	24	2.596	2.0	2.8	4.8
<i>Condalia spathulata</i>	23	--	--	--	23	1.036	2.0	1.1	3.1
<i>Berberis trifoliata</i>	10	3	--	--	13	1.267	1.1	0.8	1.9
<i>Forestiera angustifolia</i>	7	--	--	--	7	2.455	0.6	0.7	1.3
<i>Leucophyllum frutescens</i>	3	--	--	--	3	0.950	0.3	0.2	0.5
<i>Schaefferia cuneifolia</i>	3	--	--	--	3	0.710	0.3	0.0	0.3
<i>Vachellia bravoensis</i>	3	--	--	--	3	0.031	0.3	0.0	0.3
Totals	870	182	46	63	1161		100.0	100.0	200.0

Appendix I. Vascular plant species encountered at Harris Ranch near Cline, Uvalde County, Texas are listed alphabetic by families. The material was collected during five field trips to the ranch between 2002 and 2003. Collecting numbers after each name are those of D.S. Seigler, and are deposited in the herbarium of the University of Illinois (ILL). Except for the genus *Acacia* s.l. nomenclature follows Jones et al. (1997). The members of the genus *Acacia* s.l. are separated into their presently recognized genera: *Senegalia* and *Vachellia*. A few species were not collected and are listed as observed in this list.

Note: *= exotic species.

MONOCOTS

Agavaceae

Nolina texana S. Watson, 15905

Bromeliaceae

Tillandsia recurvata L., 15839

Liliaceae

Cooperia drummondii Herbert, 15462, 15722

Nothoscordum bivalve (L.) Britton, 15452

Poaceae

Aristida purpurea Nuttall var. *purpurea*, 15256

Aristida purpurea Nuttall var. *wrightii* Nash, 15241, 15431

Bouteloua barbata Lagasca y Segura, 15447

Bouteloua curtipendula (Michaux) Torrey, 15240
**Bromus catharticus* Vahl, 15249
Buchloe dactyloides (Nuttall) Engelman, 15257
Chloris cucullata Bischoff, 15429, 15433
**Cynodon dactylon* (L.) Persoon, 15222
Elymus virginicus L., 15220
Digitaria cognatum (Schultes) Pilger, 15436, 15448
Nassella leucotricha (Trin. & Rupr.) Pohl, 15242
Panicum hallii Vasey var. *filipes* (Scribner) Waller, 15434
Panicum hallii Vasey var. *hallii*, 15449, 15450
Paspalum pubiflorum Fournier, 15247
Setaria texana Emery, 15430
Tridens albescens (Vasey) Wooton & Standley, 15254, 15255
Tridens muticus (Torrey) Nash, 15234
Tridens texanus (S. Watson) Nash, 15432
Urochloa fasciculata (Swartz) Webster, 15435

DICOTS

Acanthaceae

Carlowrightii torreyana Wasshausen, 15706
Ruellia occidentalis (A. Gray) Tharp & Barkley, 15264

Anacardiaceae

Rhus aromatic Aiton, 15712
Rhus microphylla Engelman, 15713

Asclepiadaceae

Cynanchum racemosum (Jacquin) Jacquin var. *unifarum* (Scheele) Sundall, 15260
Matelea reticulata (A. Gray) Woodson, 15718

Asteraceae

Acourtia runcinata (D. Don) Turner, 15699
Acourtia wrightii (A. Gray) Reveal & King, 15704
Ambrosia confertiflora DC., 15442
Calyptocarpus vials Lessing, 15444
Chaetopappa asteroides (Nuttall) DC., 15457
Conoclinium greggii (A. Gray) Small, 15437
Dyssodia pentachaeta (DC.) Robinson, 15232, 15458
Grindelia microcephala DC., 15225
Gutierrezia texana (DC.) Torrey & A. Gray, 15842
Helenium quadridentatum Labillardière, 15246
Helianthus debilis Nuttall, 15226
Melampodium cinereum DC., 15456
Parthenium confertum A. Gray, 15236, 15454
Psilostrophe gnaphalioides DC., 15854
Ratibida columnifera (Nuttall) Wooton & Standley, 15243
Simsia calva (Engelman & Gray) A. Gray, 15441
Thelesperma burridgeanum (Regel et al.) Blake, 15235

Berberidaceae

Berberis trifoliolata Moricand, 15857, 15919

Boraginaceae

Heliotropium tenellum (Nuttall) Torrey, 15849

Tiquilia canescens (DC.) Richardson, 15701

Cactaceae

Opuntia atrispina Griffiths, 15725

Opuntia engelmannii Salm-Reifferscheid-Dyck, observed

Opuntia leptocaulis DC., 15711

Celastraceae

Schaefferia cuneifolia A. Gray, 15845

Cucurbitaceae

Ibervillea lindheimeri (A. Gray) Greene, 15846

Cuscutaceae

Cuscuta gronovii Schultes, 15453

Ebenaceae

Diospyros texana Scheele, 15698

Euphorbiaceae

Acalypha monostachya Cavanilles, 15237

Argythamnia humilis (Engelmann & A. Gray) Muell. Arg., 15253

Bernardia myricaefolia (Scheele) S. Watson, 15851

Croton incanus Kunth, 15900

Croton lindheimerianus Scheele, 15708

Croton monanthogynus Michaux., 15856

Euphorbia micromera Boissier, 15451

Phyllanthus polygonoides Sprengel, 15460

Stillingia treculiana (Muell. Arg.) I.M. Johnston, 15714

Tragia ramosa Torrey, 15705, 15721

Fabaceae

Dalea pogonathera A. Gray, 15461

Eysenhardtia texana Scheele, 15697

Indigofera miniata Ortega, 15707

Mimosa aculeaticarpa Ortega var. *biuncifera* (Bentham) Barneby, 15238

Prosopis glandulosa Torrey, 15853

Senegalia berlandieri (Bentham) Britton & Rose, 15250

Senegalia x turneri Seigler & Ebinger, 15217

Senegalia wrightii (Bentham) Britton & Rose, 15228

Senna roemeriana (Scheele) Irwin & Barneby, 15702, 15709

Sophora secundiflora (Ortega) DC., 15723

Vachellia rigidula (Bentham) Seigler & Ebinger, 15251

Vachellia bravoensis (Isely) Seigler & Ebinger, 15218

Gentianaceae

Sabatia campestris Nuttall, 15695

Lamiaceae

Hedeoma drummondii Benth, 15239b

**Marrubium vulgare* L., 15245

Salvia ballotiflora Benth, 15443

Salvia texana (Scheele) Torrey, 15239a

Malvaceae

Abutilon fruticosum Guillem. & Perrottet, 15263, 15540

**Malvastrum coromandelianum* (L.) Garcke, 15248, 15445

Sida abutifolia Miller, 15446, 15855

Menispermaceae

Cocculus carolinus (L.) DC., 15716

Nyctaginaceae

Acleisanthes longiflora A. Gray, 15715

Nyctaginia capitata Choisy, 15719

Oleaceae

Forestiera angustifolia Torrey, 15920

Onagraceae

Gaura parviflora Lehmann, 15223

Oxalidaceae

Oxalis dichondraefolia A. Gray, 15703

Passifloraceae

Passiflora tenuiloba Engelm., 15221

Phytolaccaceae

Rivina humilis L., 15852

Polygalaceae

Polygala lindheimeri A. Gray, 15694

Polygala ovatifolia A. Gray, 15439

Ranunculaceae

Clematis drummondii Torrey & A. Gray, 15244

Rhamnaceae

Colubrina texensis (Torrey & A. Gray) A. Gray, 15230

Condalia hookerii M.C. Johnston, 15231

Condalia sparthulata A. Gray, 15844

Zizyphus obtusifolia (Torrey & A. Gray) A. Gray, 15843

Rosaceae

Prunus minutiflora Engelm., 15918

Rubiaceae

Hedyotis nigrans (Lamarck) Fosberg, 15696

Rutaceae

Ptelea trifoliata L., 15261

Thamnosma texana (A. Gray) Torrey, 15901

Sapindaceae

Sapindus saponaria L. var. *drummondii* (H. & A.) L. Benson, 15841

Ungnadia speciosa Endlicher, 15850

Scrophulariaceae

Leucophyllum frutescens (Berlandier) I.M. Johnston, 15229

Solanaceae

Solanum elaeagnifolium Cavanilles, 15859

Solanum rostratum Dunal, 15692

Solanum triquetrum Cavanilles, 15438

Sterculiaceae

Hermannia texana A. Gray, 15717

Melochia pyramidata L., 15720

Ulmaceae

Celtis laevigata Willdenow, 15252

Celtis pallida Torrey, 15858

Ulmus crassifolia Nuttall, 15840

Verbenaceae

Aloysia gratissima (Gillies & Hooker) Troncoso, 15233

**Lantana camara* L., 15224

Verbena halei Small, 15459

Viscaceae

Phorodendron tomentosum (DC.) A. Gray, 15724

Zygophyllaceae

Guajacum angustifolium Engelmann, 15710

Analysis of *Juniperus phoenicea* from throughout its range in the Mediterranean using DNA sequence data from nrDNA and petN-psbM: The case for the recognition of *J. turbinata* Guss.

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ABSTRACT

DNA sequences were analyzed from 19 populations of *J. phoenicea* from throughout its range. The sequence data (nrDNA, petN-psbM) revealed that *J. phoenicea* is clearly divided into two taxa. These taxa have been recognized as var. (subsp.) *phoenicea* and var. (subsp.) *turbinata* by Adams (2011) and Farjon (2005). However, the magnitude of the differences in the DNA regions, along with the differences in pollen shedding times, morphology and prodelphinidin content support the recognition of *J. turbinata* Guss. No differentiation was found between the typical Mediterranean and Canary Island populations, offering no support for the recognition of *J. phoenicea* subsp. *canariensis* (Guyot) Rivas-Martinez. *Juniperus turbinata* appears to be widespread from Madeira - Canary Islands to the Sinai with few DNA differences among most populations. However, some populations (Grazalema, Madeira, Sinai, central Italy) had moderate amounts of divergence (3-4 mutations) and warrant additional study.

Published on-line www.phytologia.org *Phytologia* 95(2): 202-209 (May 1, 2013).

KEY WORDS: *Juniperus phoenicea* var. *phoenicea*, var. *turbinata*, *Juniperus turbinata*, phylogeny, Cupressaceae, DNA, nrDNA (ITS), petN-psbM, geographic variation.

The genus *Juniperus* is comprised of approx. 75 species in 3 sections (Adams, 2011) with serrate (denticulate) leaf-margined species found in both the eastern hemisphere (1 species) and western hemisphere (21 species). *Juniperus phoenicea* is the only serrate-leaf juniper in the eastern hemisphere and generally treated as *J. p.* var. *phoenicea* and var. *turbinata* (Adams, 2011) or as subsp. (Farjon, 2005). However, Adams and Schwarzbach (2013) have recently shown that *J. phoenicea* is not part of a clade of serrate-leaf junipers occurring in the western hemisphere, leading them to denote *J. phoenicea* as a 'pseudoserrate' juniper. In addition, they found *J. p.* var. *phoenicea* and var. *turbinata* to be as different in their DNA sequences as several other recognized species of *Juniperus*; lending support for the recognition of *J. turbinata* Guss. as proposed by Lebreton and Perez de Paz (2001) based largely on the concentration of prodelphinidin, a polymeric tannin. The prodelphinidin data suggested that *J. phoenicea* var. *phoenicea* was confined to the Iberian Peninsula (Fig. 1), with var. *turbinata* being widespread throughout the Mediterranean (Fig. 1). However, Farjon (2005) considered subsp. *phoenicea* to be widespread in the Mediterranean and subsp. *turbinata* to be confined to littoral maritime habitats (sand and rocks). Adams (2011) followed the distributions of Farjon (2005), except for the Canary Islands and Madeira, which, based on DNA sequence data, have been shown to be var. *turbinata* (Adams et al. 2010).

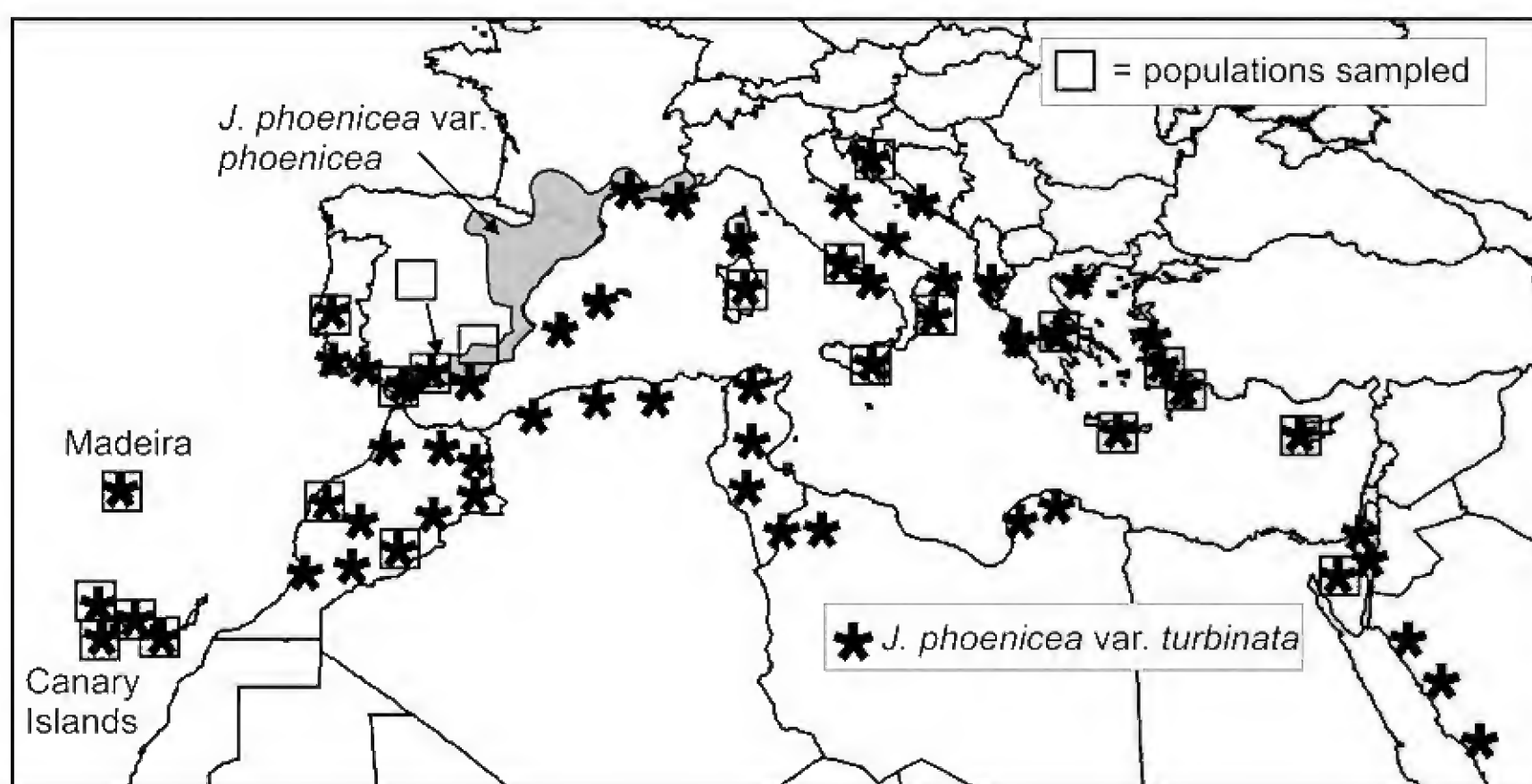


Figure 1. Distribution of *J. phoenicea* (adapted from Lebreton and Perez de Paz (2001) and Adams et al. (2010)). Squares show populations of vars. *phoenicea* and *turbinata* sampled in the present study.

Arista and Ortiz (1995) analyzed plants from Sierra de Grazalema, Spain and reported the seed cones of subsp. *turbinata* were larger (7.5 mm L x 8.8 mm W) than subsp. *phoenicea* (6 mm L x 5.8 mm W) at that location. Arista, Ortiz and Talavera (1997) analyzed the reproductive isolation of subsp. *phoenicea* and *turbinata* in the Sierra de Grazalema populations. They reported flowering (pollen shedding) occurred in the fall (Oct.-Nov.) for subsp. *turbinata* and in the spring (Feb.-March) for subsp. *phoenicea*, effectively preventing cross-pollination in these (normally) monocious taxa. Interestingly, subsp. *phoenicea* grew on dolomitic soil whereas subsp. *turbinata* was found on Cambrian limestone (in contrast to coastal sand dunes in southern Spain).

Mazur et al. (2003, 2010) compared several populations of var. *turbinata* (Portugal, sw Spain, Italy and Morocco) and of var. *phoenicea* from ne Spain using 14 morphological characters. They did not find the seed cone length or width to be very different, but the ratio of cone length/width (i.e., shape) seemed to discriminate between the taxa. Perhaps the best character (Mazur et al. 2003) was the number of seeds/cone (4.97 - 5.96 for var. *turbinata* vs. 8.05 for var. *phoenicea*).

Table 1. Morphological differences between var. *phoenicea* and var. *turbinata*.

	var. <i>phoenicea</i>	var. <i>turbinata</i>
seed cones	spherical to globular	elongate or turbinate (when immature) almost spherical (when mature) in some populations.
number of seeds	smaller, 5-9 mm long more, 3(7-9)13	larger, 7-11 mm long fewer, 3(4-7)10
pollen shed	spring (Feb. - March)	fall (Oct.-Nov.)
branchlets	thicker	thinner
branchlets bark	gray to brown	reddish
habitat	dolomitic soil	sand, Cambrian limestone, volcanic rock

Adams et al. (2002) utilized RAPDs to compare *Juniperus phoenicea*, *J. p.* var. *canariensis*, *J. p.* subsp. *eu-mediterranea*, and *J. p.* var. *turbinata* from El Peñón, Spain, Setubal, Portugal, Corse (from high and low α -pinene plants), the Canary Islands (Tenerife), Nea Epidavios, and Delphi, Greece, and the Tarifa sand dunes, Spain. They found the high and low α -pinene plants from Corse clustered together, along with other var. *turbinata* populations. The var. *phoenicea* plants from El Peñón, Spain formed a separate cluster, with all the other populations clustering with var. *turbinata* from the Tarifa sand dunes, Spain (Adams et al., 2002). They concluded that *J. p.* var. *canariensis* and *J. p.* subsp. *eu-mediterranea* were not distinct taxa but included in *J. p.* var. *turbinata*.

A second study with RAPDs data (Adams et al., 2006) compared *J. phoenicea* from the Canary Islands with plants of var. *turbinata* from Morocco and the Tarifa sand dunes, Spain plus var. *phoenicea* from El Peñón, Spain. They found the plants from the Canary Islands, Morocco, and Tarifa sand dunes clustered together, whereas var. *phoenicea* from El Peñón, Spain formed a separate cluster.

Dzialuk et al. (2011) also used RAPDs to compare plants from Andorra, France, Morocco, Portugal and 3 sites in Spain. Principal coordinates clearly separated 3 of the subsp. *phoenicea* populations from subsp. *turbinata* (Fig. 2, Dzialuk et al., 2011), but one population of putative subsp. *phoenicea* from Spain (SP 3) was ordinated with populations of subsp. *turbinata*. Interestingly, previous work (Boratynski et al., 2009), using isozyme data, found SP 3 to cluster with other populations of subsp. *phoenicea* from Spain. In fact, Boratynski et al. (2009, Fig. 3) showed all populations of subsp. *phoenicea* from Spain and France clustered together and populations of subsp. *turbinata* from Greece, Italy, Morocco, Portugal, Spain and Turkey formed a separate cluster.

The purpose of the present study was to examine DNA sequence data from nrDNA and petN-psbM regions for individuals of *J. phoenicea* from throughout its range, to determine if the two taxa are distinct and if they are distributed as suggested by the prodelphinidin data of Lebreton and Perez de Paz (2001), see Fig. 1 above.

MATERIALS AND METHODS

Specimens used in this study: **var. *phoenicea*:**

Spain, El Peñón, 37° 35' 38" N, 3° 31' 22" W, elev. 760m, Adams 7077-7079,
Spain, Sierra de Grazalema, 36° 47' 51.5" N, 5°24' 43.7"W, 835 m; *M. Arista* 1-5, Baylor specs. Adams 13813-13817.

var. *turbinata*:

Canary Islands, Tenerife, 0.5km S.of Tejina de Isora on rt.822, 29° 10' 48"N, 16° 45' 53"W, ca. elev. 520m, Adams 8147-8149

Corse, France, *Joe Casanova* 1-3, *Adams* 8893-8895,
 Crete, Dragonada Isl., 35° 22' 32" N; 26° 11' 01" E. elev. ca. 30 m, *Avramakis Manolis* 1-2, Baylor specs. *Adams* 13605-13606,
 Croatia, Ugljan Island, 44° 05' 0.27" N, 15° 09' 39.29"E, elev. 20-32 m, *Zlato Liber* 1-5, Baylor specs. *Adams* 13589-13593,
 Cyprus, CYP-1 35° 00' N, 32° 18' E elev. 400 m, *Adam Boratynski CYP-1(1-5)*, Baylor specs. *Adams* 13351-13355,
 Cyprus, CYP-2 34° 58' N, 34° 04' E, elev. 20 m, *Adam Boratynski IT-1(1-5)*, Baylor specs. *Adams* 13356-13360
 Italy, central, Sabaudia, 41 ° 15' N, 13 ° 02" E, elev. 10 m, *Adam Boratynski IT-1(1-5)*, Baylor specs. *Adams* 13336-13340,
 Italy, southern, Crotone, 38° 53' 36" N, 17° 05' 42" E, elev. 10 m, *Adam Boratynski IT-2(1-5)*, Baylor specs. *Adams* 13341-13345,
 Madeira Island, Portugal, elev. ca. 20m, *Adams* 11502-11504,
 Morocco, rd to Oukaimeden, 31° 21.033'N, 07° 45.893'W, elev. 940m, *Adams* 9408-9410
 Morocco, Essaouria sand dunes, 31° 29' 26"N, 9° 44' 29" W, elev. 98m, *Adams* 10407-10408, (ex *Nadia Achak*),
 Portugal, Setubal, *Adams* 7074-7076,
 Sicily, near Piano Pirrera near Acate (Ragusa), 37° 01' 35.75" N; 14° 26' 07.86" E., 120 m, *Pietro Minissale & Saverio Sciandrello* 1-5, Baylor specs. *Adams* 13778-13782
 Sinai, 30°38'09"N, 33°26'53"E, elev. 700 m *Hagar Leschner* 1-5, Baylor specs. *Adams* 13495-13499,
 Spain, Sierra de Grazalema, 36° 48' 10.9"N, 5° 24' 21.2"W, elev. 829m, *M. Arista* 6-10, Baylor specs. *Adams* 13818-13822,
 Spain, Tarifa sand dunes, elev. ca. 20m, *Adams* 7202-7204,
 Turkey, Orak Island, Bodrum-Mugla Province, 36° 58' 25"N, 27° 35' 45" E, elev. 44 m, *Tugrul Mataraci T-1*, Baylor specs. *Adams* 12397,
 Turkey, Marmaris Peninsula, 36° 49' N, 27° 50' E, elev. 700 m, *Adam Boratynski Tu-1(1-5)*, Baylor specs. *Adams* 13346- 13350,
***Juniperus sabina* (outgroup):** Switzerland, Baltschieder, 1300m, *Adams* 7611-7612,
 Voucher specimens are deposited at BAYLU herbarium Baylor University.

One gram (fresh weight) of the foliage was placed in 20 g of activated silica gel and transported to the lab, thence stored at -20° C until the DNA was extracted. DNA was extracted from juniper leaves by use of a Qiagen mini-plant kit (Qiagen, Valencia, CA) as per manufacturer's instructions.

Amplifications were performed in 30 µl reactions using 6 ng of genomic DNA, 1.5 units Epi-Centre Fail-Safe Taq polymerase, 15 µl 2x buffer E (petN, trnD-T, trnL-F, trnS-G) or K (nrDNA) (final concentration: 50 mM KCl, 50 mM Tris-HCl (pH 8.3), 200 µM each dNTP, plus Epi-Centre proprietary enhancers with 1.5 - 3.5 mM MgCl₂ according to the buffer used) 1.8 µM each primer. See Adams, Bartel and Price (2009) for the ITS and petN-psbM primers utilized. The primers for trnD-trnT, trnL-trnF and trnS-trnG regions have been previously reported (Adams and Kauffmann, 2010).

The PCR reaction was subjected to purification by agarose gel electrophoresis. In each case, the band was excised and purified using a Qiagen QIAquick gel extraction kit (Qiagen, Valencia, CA). The gel purified DNA band with the appropriate sequencing primer was sent to McLab Inc. (San Francisco) for sequencing. Sequences for both strands were edited and a consensus sequence was produced using Chromas, version 2.31 (Technelysium Pty Ltd.) or Sequencher v. 5 (genecodes.com). Sequence datasets were analyzed using Geneious v. R6-1 (Biomatters. Available from <http://www.geneious.com/>) and the MAFFT alignment program. Further analyses utilized the Bayesian analysis software Mr. Bayes v.3.1 (Ronquist and Huelsenbeck 2003). For phylogenetic analyses, appropriate nucleotide substitution models were selected using Modeltest v3.7 (Posada and Crandall 1998) and Akaike's information criterion.

Minimum spanning networks were constructed from mutational events (ME) data using PCODNA software (Adams et al., 2009; Adams, 1975; Veldman, 1967).

RESULTS AND DISCUSSION

Sequencing nrDNA (nuclear ribosomal DNA) ITS regions yielded 1276 bp of data. Sequencing petN-psbM (intergenic region of chloroplast DNA) provided 855 bp of data. Combined, these data afforded 2131 bp of data. A Bayesian tree (with *J. sabina* as an outgroup) shows (Fig. 2) strong support for var. *phoenicea* and var. *turbinata* as previously reported (Adams and Schwarzbach, 2013). Most of the var. *turbinata* populations displayed very little variation. Exceptions were: one plant from Delphi, Greece which is in a distinct clade with Cyprus plants; whereas the other Delphi plant is in another clade; and the plants from Sinai, Madeira and Grazalema are separate from the major clade of var. *turbinata* (Fig. 2).

To examine the magnitude of the differences among the populations, a minimum spanning network was constructed (Fig. 3). The outgroup (*J. sabina*) is quite distant (65 MEs, Fig. 3) as previously shown by Adams and Schwarzbach (2013). In fact, *J. phoenicea* is not close to any juniper species and certainly not related (unless very distantly) to the serrate-leaf junipers of North America.

Notice that the minimum spanning link between var. *phoenicea* and var. *turbinata* is 13 MEs, based on data from only 2 DNA sequences. This is a very large difference, comparable to species differences in section *Sabina* (Adams and Schwarzbach, 2012). Many of the populations of var. *phoenicea* differ by only 0 or 1 MEs (Fig. 3). A few of the populations differ from the central group by 4 MEs: Grazalema, Spain; central Italy; Madeira Island and Sinai.

There is some variation in var. *phoenicea* with plants at El Peñón differing by 2 MEs and the Grazalema plants are separated by 3 MEs from nearby El Peñón (Fig. 3).

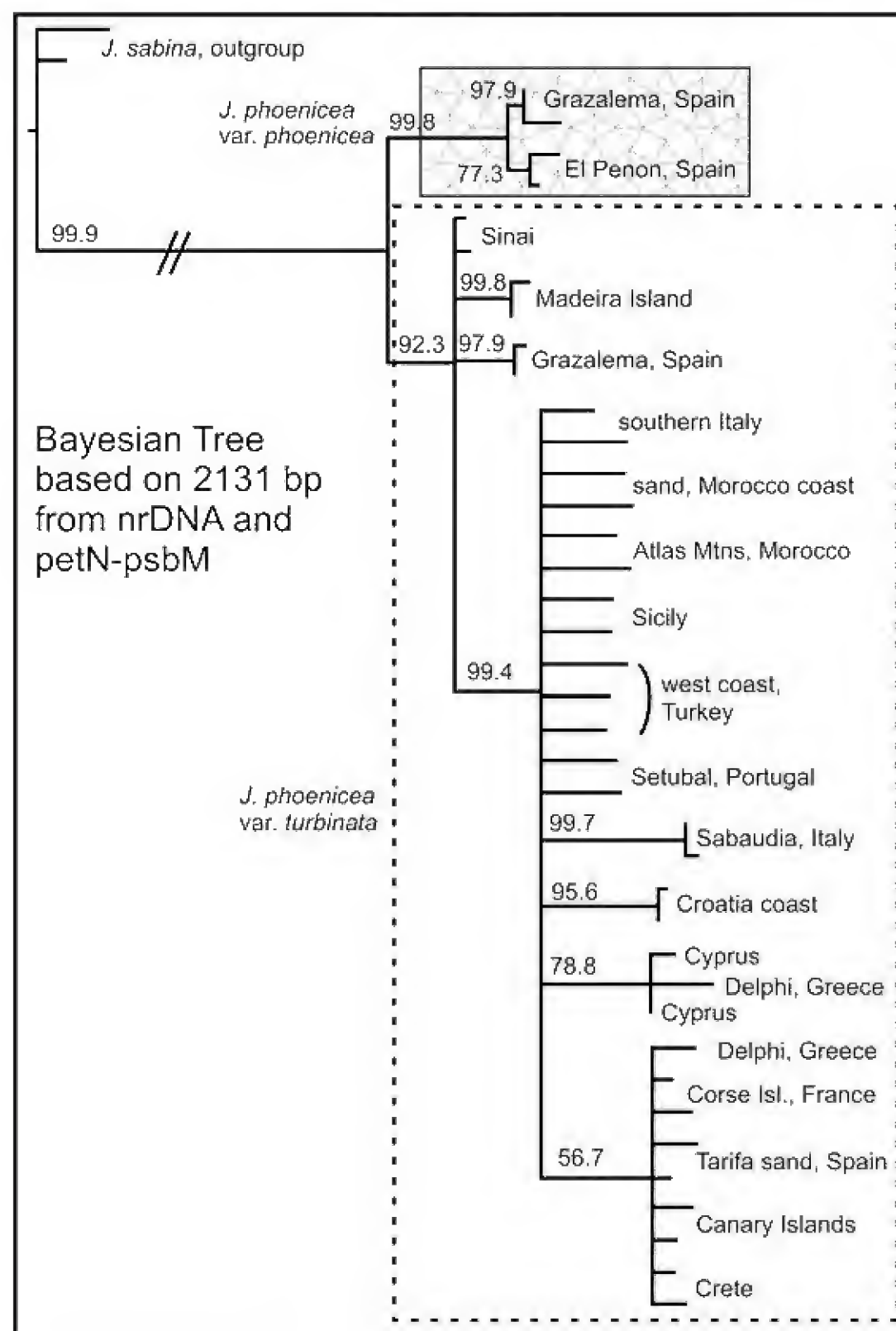


Figure 2. Bayesian tree based on nrDNA and petN-psbM sequences. The numbers at the branches are posterior probabilities (as percents).

Plotting the minimum spanning network onto a geographic map offers additional perspective (Fig. 4). The major feature of the network is the nearly identical DNA sequences (i.e., 0, 1 or 2 MEs) between most of the populations. The Sicily population, particularly representative as it is probably the largest on the island (Minissale and Sciandrello 2013), appears to be the most central of the nodes with no (0) MEs to Morocco and southern Italy, and by only 1 ME to Tarifa, Spain and Turkey populations. In addition, the Canary Islands population had no differences from the Tarifa, Spain population (Fig. 4).

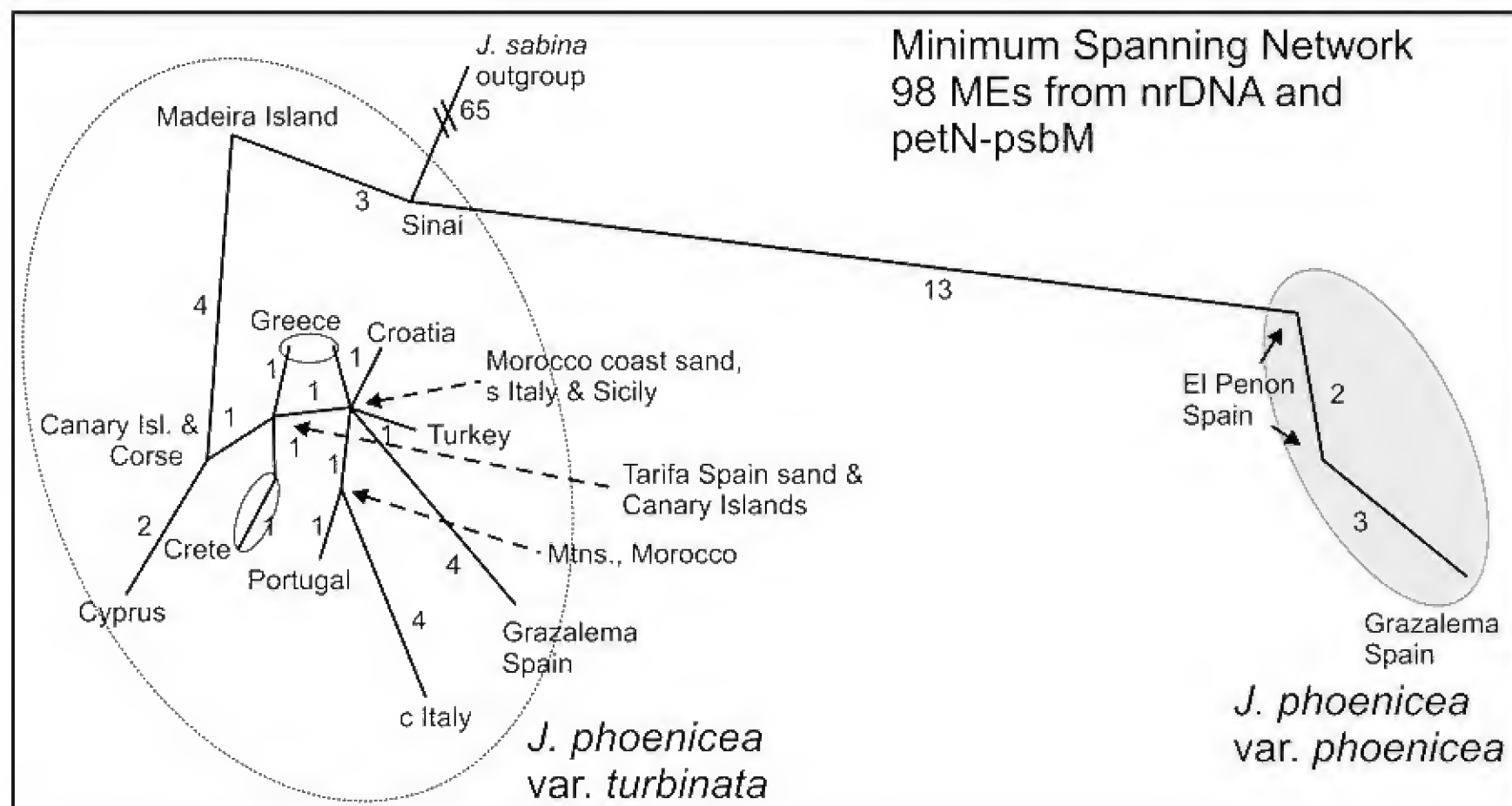


Figure 3. Minimum spanning network based on nucleotide substitutions and indels (Mutational Events, MEs). Numbers next to links are the number of MEs.

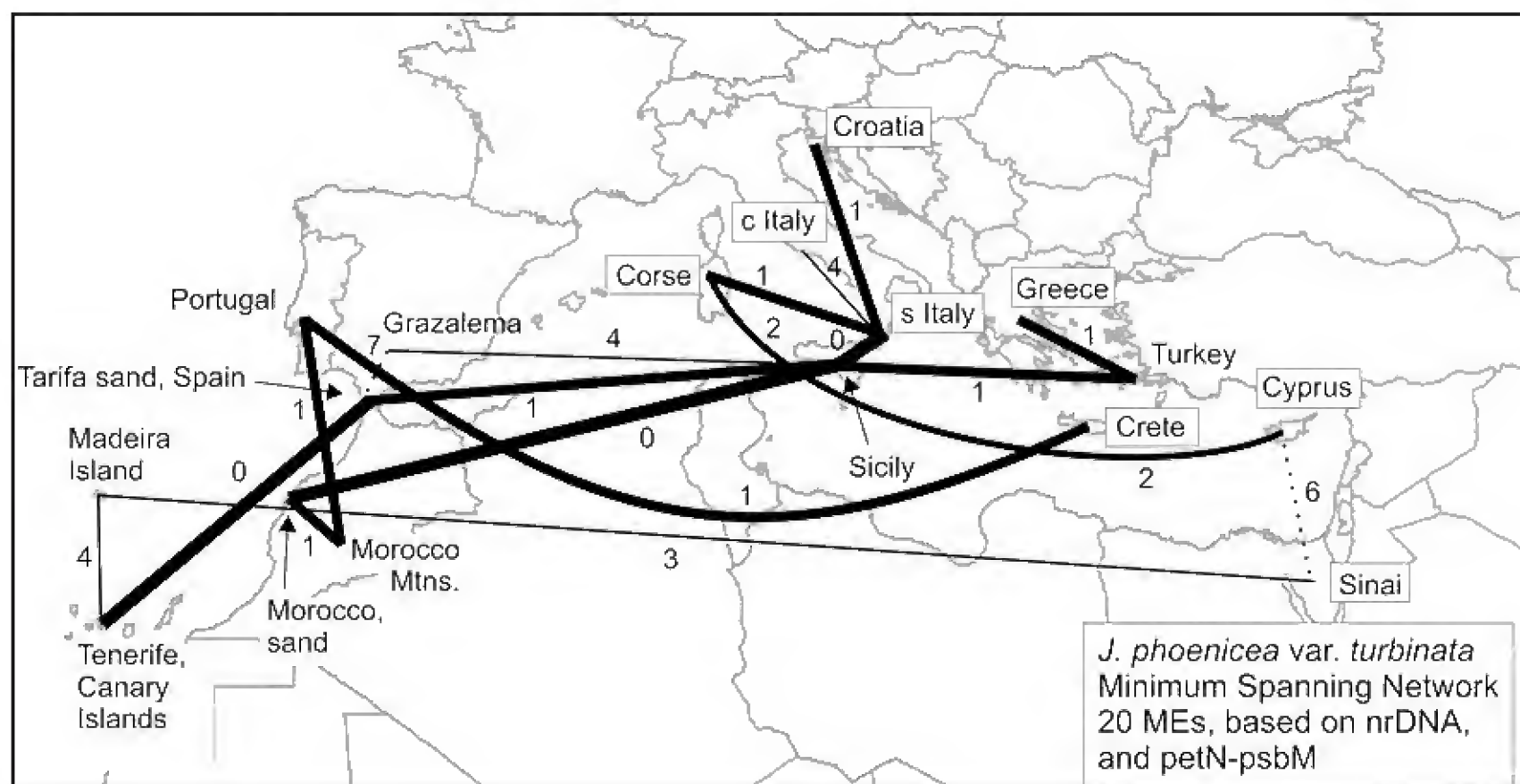


Figure 4. Minimum spanning network plotted onto a geographic map. Numbers next to lines are the number of MEs for the link. The width of a line is proportional the similarity between nodes. The widest lines denote no differences (0 MEs), whereas the narrowest lines show the least similar nodes (3 or 4 MEs). Dotted lines are links to the nearest geographic population.

Several populations have 3 or 4 ME differences from their nearest node. Grazalema, Spain differs by 4 MEs from Sicily (its nearest or most similar neighbor) and by 7 MEs to the nearby Tarifa sand population (Fig. 4). Because both var. *phoenicea* and var. *turbinata* co-occur at the Grazalema site, one might suspect that hybridization may be the cause of the unusual differentiation in var. *turbinata*. But, because they shed pollen in the spring and fall, respectively, that should not be a factor. The nrDNA (ITS) sequences for var. *phoenicea* and var. *turbinata* from Grazalema differ by 6 nucleotide substitutions and 4 indels. If hybrids are involved, one would expect to find some of the substitution differences to be polymorphic. However, re-examination of the sequencing chromatograms of these plants failed to reveal heterozygous peaks, implying that hybridization is not involved in the divergence of the var. *turbinata* at Grazalema from other populations.

Madeira Island plants differ by 4 MEs from the Canary Islands plants and 3 MEs from the Sinai plants (Fig. 4). The DNA differences for Madeira - Canary Islands parallel the differences found in *Juniperus cedrus* from Madeira and Canary Islands (9 MEs, nrDNA + petN-psbM, Fig. 7, Adams et al., 2010). Combined with leaf terpenoid differences, Adams et al. (2010) recognized *J. maderensis* on Madeira. However, the present differences (4 MEs in nrDNA + petN-psbM) are not as great, so it is premature to recognize the Madeira var. *turbinata* as a different variety. The divergence of the small population on Madeira may be the results of a founder event or genetic drift. Additional research on leaf terpenoids (in progress) may help resolve this taxonomic question.

It is interesting that the Sinai population is most closely linked to Madeira (3 MEs, Fig. 4), but it is 6 MEs distant from the nearby Cyprus population. It seems improbable that seeds were transported between the Sinai and Madeira populations. Perhaps research on leaf terpenoids (in progress) will help illuminate this problem.

Finally, the central Italy (Sabaudia) population differs by 4 MEs from the southern Italy plants. Boratynski et al. (2009) included the central Italy population (IT-1) in their study using isozymes. They found it to cluster closely with Greece (GR-1) and Morocco (MOR). The central Italy population is on old coastal sand dunes. The population is large and not too distant from other populations in Italy, Sicily, Corse and Croatia (Figs. 1, 4). Perhaps variation of 3-4 MEs might be expected. Additional research is needed.

CONCLUSION

In summary, this study indicates that *J. phoenicea* is clearly divided into two taxa. These taxa have been recognized as var. (subsp.) *phoenicea* and var. (subsp.) *turbinata* by Adams (2011) and Farjon (2005). However, the magnitude of the differences in the DNA regions sequenced in this and Adams and Schwarzbach (2012), along with the differences in pollen shedding times, morphology and prodelphinidin (Lebreton and Perez de Paz, 2001) support the recognition of *J. turbinata* Guss. No differentiation was found between the typical Mediterranean and Canary Island populations, offering no support for the recognition of *J. phoenicea* subsp. *canariensis* (Guyot) Rivas-Martinez.

Juniperus turbinata appears to be widespread from Madeira - Canary Islands to the Sinai with few DNA differences among most populations. However, some populations (Grazalema, Madeira, Sinai, central Italy) show moderate amounts of divergence (3-4 mutations) and deserve additional study.

ACKNOWLEDGEMENTS

Thanks to Saverio Sciandrello for collecting assistance (Sicily) and Tonnie Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Revised key to *Hymenostephium* of Mexico with addition of *H. superaxillare*

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ABSTRACT

In my recent recension of the genus *Hymenostephium* for Mexico (Turner 2013), I inadvertently published an incomplete account that lacked the species, *H. superaxillare*, failing to include the taxon in the key of the text. A complete accounting of the species is provided here, along with a revised key and a map showing its distribution. Published on-line www.phytologia.org *Phytologia* 95(2): 210-211 (May 1, 2013).

KEY WORDS: Asteraceae, *Hymenostephium*, *H. superaxillare*, Mexico Chihuahua, Durango.

In my very recent paper, Recension of the Mexican species of *Hymenostephium* (*Phytologia* 2013: 95:1-9), I failed to include in this **H. superaxillare** (having carelessly submitted an earlier version of the paper). I provide here an amended key, an account of the errant species, and a map showing its distribution.

Key to species

1. Tap-rooted annuals, or erect, simple-stemmed, perennials, 20-80 cm high...(4)
1. Suffruticose herbs, or recumbent shrubs 1-4 m high...(2)
2. Heads relatively small, 4-5 mm high, 2-3 mm wide (rays excluded); disc florets 5-10; leaves glabrous, or nearly so; oak forests, Mic, Gue.....**H. hintonii**
2. Heads relatively large, 4-7 mm high, 4-10 mm wide; disc florets mostly 12 or more; leaves variously pubescent....(3)
3. Involucres 8-10 mm across; ray florets 11-13; s Chi, n Sin.....**H. superaxillare**
3. Involucres 4-6 mm across; ray florets 5-8; widespread.....**H. cordatum**
4. Perennial herbs 20-80 cm high, arising from a corm-like base; achenes pubescent, epappose; Nay, Jal.....**H. websteri**
4. Annual herbs 20-80 cm high, arising from slender tap-roots; achenes otherwise...(5)
5. Outer involucral bracts mostly in 1-2 series, grading into the inner bracts...(7)
5. Outer involucral bracts elliptic-ovate, exactly 5, in a single whorl...(6)
6. Leaves mostly 1-2 cm long, 0.2-0.8 cm wide; involucres ca 2 mm high; Mic..**H. woronowii**
6. Leaves mostly 3-6 cm long, 1-4 cm wide; involucres 4-5 mm high; Mex, Mor, Gue
.....**H. uniseratum**
7. Involucres 2-4 mm high; achenes glabrous; Mic**H. woronowii**
7. Involucres 4-12 mm high; achenes pubescent...(8)
8. Leaves sessile or nearly so.....**H. tenuis**
8. Leaves with well-defined petioles 3-15 mm long; Pacific shore lines, se Oax, sw Cps
.....**H. gracillimum**

HYMENOSTEPHIUM SUPERAXILLARE Blake, Proc. Biol. Soc. Washington 37: 57. 1924.

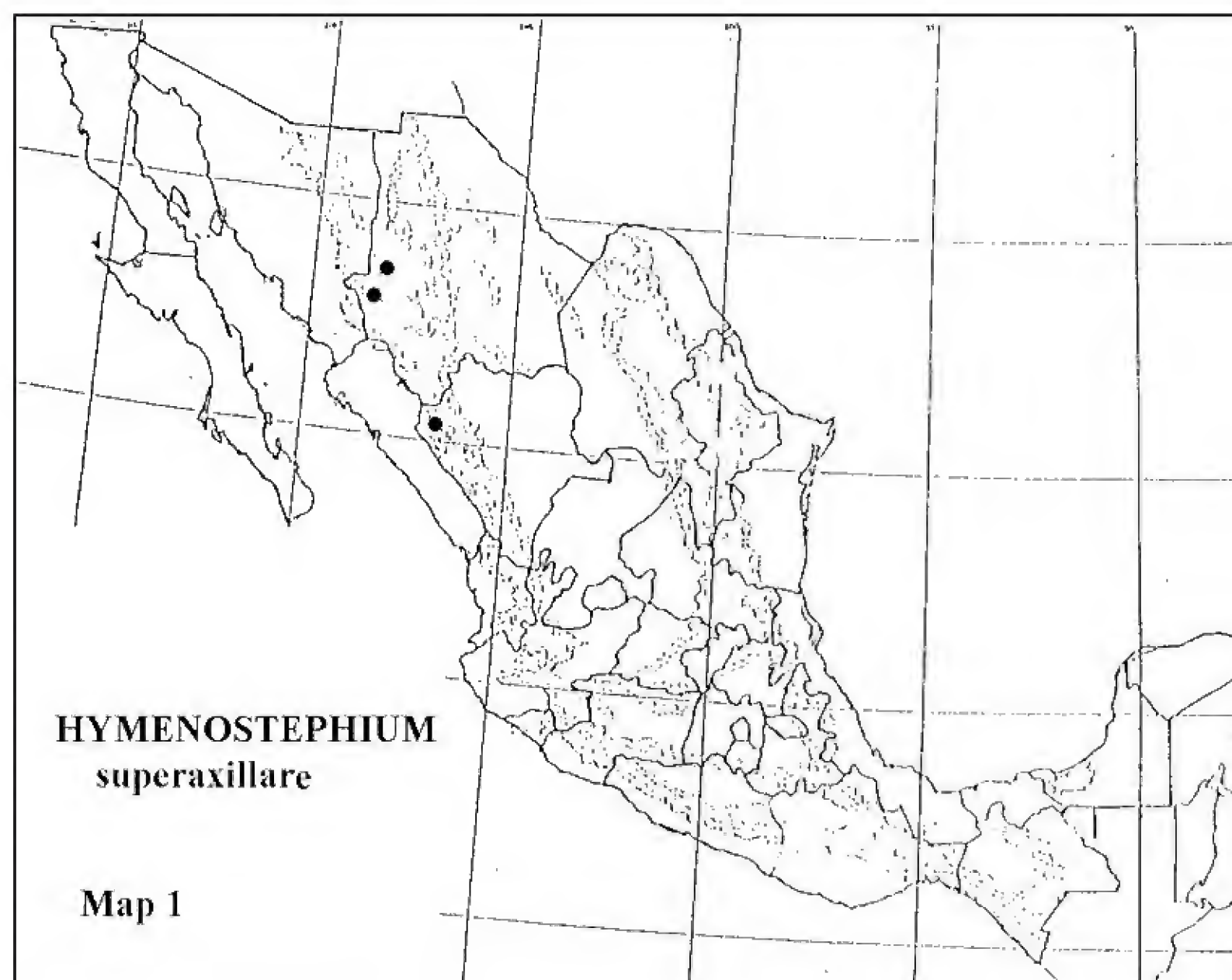
Viguiera superaxillaris (Blake) B.L. Turner

Viguiera vorobikae B.L. Turner

sw Chi and nw Dur, pine-oak forests, 600-2000 m; Oct-Nov. **Map 1**

Shrub 1-3 m high. **Leaves** ovate, 7-12 cm long, 3-5 cm wide; petioles 1-2 cm long; blades scabrous-pubescent above and below, the margins crenulo-dentate. **Capitulescence** of 2-3 terminal heads, the ultimate peduncles scabrous-pubescent, 3-7 cm long. **Involucres** hemispheric, 3-4 seriate, 5-6 mm high, 8-10 mm across; bracts ovate-lanceolate, subequal, the outer series somewhat foliaceous and reflexed. **Ray florets** 11-13, neuter; corollas yellow, the ligules 8-12 mm long, 3-5 mm wide. **Disc florets** numerous, yellow, ca 4 mm long; tube ca 1 mm long, the limb ca 3 mm long. **Anthers** brown, ca 2 mm long, the filaments glabrous. **Achenes** black, epappose, 2.5-2.8 mm long, ca 1 mm wide.

As noted by Blake in his original description, "This species has the largest heads of any known *Hymenostephium*, and is further distinguished by its phyllaries, which are broader than in any other species and do not have the attenuate or very narrowly acuminate tips found in practically all the others." He aptly notes that it appears nearest the epappose forms of *H. cordatum*. My description of *Viguiera vorobikae* was based upon specimens clearly referable to *Hymenostephium superaxillare*, the error corrected soon after its needless description (cf. Turner, 1990. Phytologia 68: 14-19).



Taxonomy of the *Argemone fruticosa* complex (Papaveraceae)

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ABSTRACT

The *Argemone fruticosa* complex, as interpreted here, is composed of two species, *A. fruticosa* and *A. turnerae*, the latter having two varieties. Reasons for the recognition of the taxa, and maps showing their distributions, are presented. Published on-line www.phytologia.org *Phytologia* 95(2): 212-214 (May 1, 2013).

KEY WORDS: Papaveraceae, *Argemone*, *A. fruticosa*, *A. ownbeyana*, *A. turnerae*, Mexico, Chihuahua, Coahuila

Argemone fruticosa Thurber ex A. Gray was first described in the year 1853, based upon a Thurber collection from the Mountain Pass of La Pena, near the village of Parras, Coahuila. As noted by Ownbey (1958), in his monograph of the genus, the species is “a local endemic and, although it has been known for over 100 years, adequate collections have never been distributed.” Indeed, he commented further that it was one of only two species (from among 23) in the genus that he had not personally studied in the field. Ownbey cited 5 herbarium collections as having been examined, including the type. Since Ownbey’s account, to my knowledge, only ca 5 additional collections (LL-TEX) have come to the fore, all from southernmost Coahuila, mostly from near Parras (Map 1).

Argemone turnerae A.M. Powell (named for the present author’s second wife, Pollie Turner) was described in 1972 by my Academic son, Mike Powell, largely because it was an edaphic endemic (occurring in gypsaceous soils) and had a combination of characters not called to the fore by Ownbey’s key to taxa, namely espinose fruits and white or whitish petals. Actually, had he examined plants of *A. fruticosa*, not available to him at the time, he would certainly have recognized its close relationship to the latter. Regardless, subsequent collections of what appeared to be *A. turnerae*, all having markedly spinulose stems and foliage, as well as semi-spinose ovaries, were annotated by the present author as *A. turnerae* var. *hispidula* B.L. Turner (unpublished), the rank given because the infraspecific taxon graded into its allopatric cohort var. *turnerae*.

Johnston (1976) selected the unpublished type of my var. *hispidula* as the type of his new species, *Argemone ownbeyana* M.C. Johnston. He was also the first to recognize its relationship to *A. fruticosa*, aptly noting:

Morphologically *A. Ownbeyana* is clearly similar to *A. fruticosa* A. Gray of southern Coahuila and to *A. turnerae* A. M. Powell of east central Chihuahua. These three taxa share a shrubby habit, tough, unlobed, glaucous leaves, large yellow-centered flowers, and shortly conic-ovoid capsules, and they stand clearly apart from the rest of the genus in these characters. They are all desert gypsophiles. They are morphologically distinguished, inter se, on the basis of their armature, a character that normally would be sufficient, along with geographic segregation, to permit the recognition of subspecies or varieties at most. However, I am led to the present conservative treatment by the strong geographic disjunction of all three and the discovery that *A. fruticosa* and *A. Turnerae* are quite distinct in their alkaloid-content (Stermitz, et al., 1973, both papers). The alkaloids of *A. Ownbeyana* have not been investigated as yet.

Johnston, at the time, also examined the following collections of *A. turnerae*, to which he appended the name *A. ownbeyana*, both of these subsequently annotated by me as intermediates to var.

hispidula; **Mexico. Chihuahua:** 9.5 km S of Ojinaga, *Johnston et al.* 10732 (LL); Gypsum hills, NE shores of Lake Gravelo on the Rio Conchos. *Powell* 2446 (TEX).

Finally, it should be noted that Schwarzbach and Kadereit (1999), using DNA data, recognized the close relationship of the present complex, stating “*A. fruticosa*/*A. turnerae* form a well-supported clade separate from *A. subintegrifolia* and sister to all other *Argemone* species.” Schwarzbach (by annotation) also accepted the infraspecific taxa that I proposed, treating these as subspecies instead of varieties, applying the name subsp. *ownbeyana* instead of that adopted here, the latter never formally published, although she used the category in her publication with Kadereit. In short, the latter authors applied the name *A. turnerae* subsp. *ownbeyana* to what I called var. *hispidula*, neither infraspecific name justified by formal publication, which is the purpose, in part, of the present paper.

Key to the *Argemone fruticosa* complex

1. Petals more nearly yellow; southern Coahuila.....**A. fruticosa**
1. Petals more nearly white; northeastern Chihuahua...(2)
2. Fruits spinose; stems and leaves hispid.....**A. turnerae** var. **hispidula**
2. Fruits w/o spines; stems and leaves not hispid.....**A. turnerae** var. **turnerae**

ARGEMONE FRUTICOSA Thurber ex A. Gray, Pl. Nov. Thurb. 306. 1854.

As noted above this is a well known taxon, occurring mainly in gyp soils of southern Coahuila in the Sierra del Alamos, Sierra del Venado and Sierra La Paila, 800-1500 m (Map 1). The species is represented by 5 collections at LL-TEX, flowering Jun-Sep, the petals reportedly yellow. Ownbey (1958) provided a detailed description of the taxon.

ARGEMONE TURNERAE A.M. Powell, Southwest. Naturalist 17: 106. 1972.

var. **turnerae**

This taxon is readily recognized by traits given in the above key; it is represented by 8 sheets at LL-TEX, 6 of these typical, and 2 intermediate to var. *hispidula*, as cited above (these not called to the fore as intermediates by Johnston).

var, **hispidula** B.L. Turner, var. and stat. nov.

Argemone ownbeyana M.C. Johnston, Wrightia 5: 259. 1976.

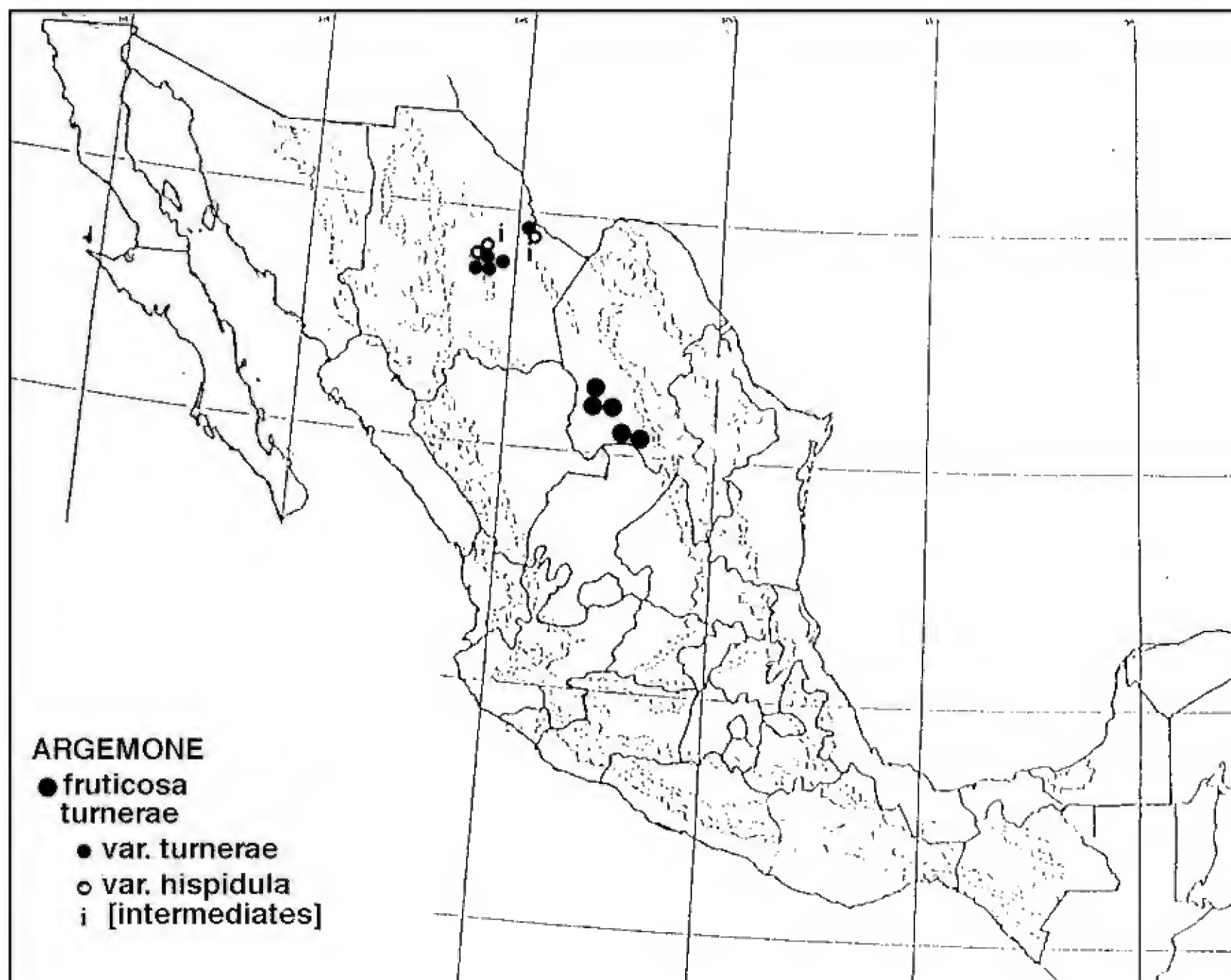
As noted above, Johnston considered treating the above three taxa at the infraspecific level, but opted for specific treatment because of the “strong geographic distribution of all three,” this not so for the varietal taxa, as indicated in Map 1. It should be noted that I could have adopted the name, var. *ownbeyana* for the present taxon, but I find the double eponymy distracting and not biomorphologically informative; besides, I had already annotated (in 1981) all of the sheets *A. ownbeyana* at LL-TEX as var. *hispidula*.

ACKNOWLEDGEMENTS

My close companion, Jana Kos, edited the paper, for which I am grateful. Distribution maps are based upon specimens on file at LL-TEX.

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Juniperus communis* var. *kelleyi*, a new variety from North America*Robert P. Adams**Biology Department, Baylor University, Box 97388, Waco, TX
76798, USA email Robert_Adams@baylor.edu**ABSTRACT**

Recent molecular analysis of *Juniperus communis*, world-wide (Adams and Schwarzbach, 2012), has shown that taxa referred to as *J. c.* var. *saxatilis* in the eastern hemisphere and in North America are not the same taxon, but are found in two distinct clades. The name is correctly applied to the taxon in the eastern hemisphere, but the taxon referred to as var. *saxatilis* in North America is given a new name: ***Juniperus communis* var. *kelleyi* R. P. Adams var. nov.** in honor of a former student, Walter A. Kelley. Leaf terpene data is presented for *J. c.* var. *kelleyi* and other *J. communis* varieties as well as *J. jackii*. The leaf oil of var. *kelleyi* is dominated by α -pinene (56.5%), δ -3-carene (11.5%) and β -pinene (5.4%) and is very similar to the oil of *J. c.* var. *depressa*. Published on-line www.phytologia.org *Phytologia* 95(3): 215-221 (August 1, 2013).

KEY WORDS: *Juniperus communis* var. *kelleyi* var. nov. , nomenclature, DNA, leaf terpenes.

Recently, Adams and Schwarzbach (2012) have shown that the North American taxon referred to as *Juniperus communis* var. *saxatilis* Pall. is not in the clade with *J. c.* var. *saxatilis* from Europe and Central Asia, but is actually most closely related to *J. c.* var. *depressa* from North America. To reflect these relationships, a new variety of *Juniperus communis* is described:

***Juniperus communis* var. *kelleyi* R. P. Adams, var. nov.** Fig. 1

Type: USA, Idaho, Blaine Co., on shore of Little Redfish Lake, 44° 09.588' N, 114° 54.372' W, 1997m, Adams 10892 (HOLOTYPE: BAYLU).

Shrubs, similar to *J. communis* var. *depressa*, but differing in having curved to slightly curved leaves, with cross section concave and stomatal band 1.5- 2 x width of green leaf margins, leaf blades free, 30° to 80° to the stem, seed cones about as long as leaves, seed cones ovoid, seed cones purple-blue when mature.

Other specimens studied: TOPOTYPES: Adams 10890, 10891, 10893, 10894 at BAYLU.

Juniperus communis var. *kelleyi* is common in the northwestern United States and B C, Canada (Fig. 2). In British Columbia and Alaska, var. *kelleyi* and var. *depressa* appear to intergrade.

The new variety is named in honor of my former student, Walter A. Kelley, Ph. D. 1976, Colorado State University. Walt passed away, unexpectedly with a heart attack in Costa Rica, Dec. 31, 2010, while on one of his many trips to the rainforest with his wife, Jan. Walt worked on isozymes of *Juniperus*. Photo (right) shows Walt collecting samples of *J. saltillensis* (Nuevo Leon, Mexico) on a trip with Tom A. Zanoni and RPA. Walt's keen interest in plants and sense of humor will be missed.





Figure 1. Holotype of *Juniperus communis* var. *kelleyi*, Adams 10892



Figure 2. Distribution of *Juniperus communis* var. *kelleyi*.

Table 3. Comparison of the leaf morphology of *J. communis* var. *kelleyi*, *J. c.* var. *saxatilis* and *J. jackii*.

	<i>J. c.</i> var. <i>kelleyi</i>	<i>J. c.</i> var. <i>depressa</i>	<i>J. jackii</i>
Stomatal band width vs. green leaf margin (GM)	1.5 - 2x GM	1-1.5x GM	2-4x GM
Leaf cross-section	concave	very concave	concave,
Leaf shape	curved	straight	curved, boat shaped
Leaf blades	free, 30° to 80°	free, 45° to 20°	mostly appressed to stem
Mature seed cones vs. leaf length	cones about as long as leaves	cones much shorter than leaves	cones as long as or longer than leaves
Seed cone shape	ovoid	ovoid	elongated oval (ellisoid) esp. in immature cones

The phylogenetic position of *J. communis* var. *kelleyi* is shown in Figure 3, where it is in a clade with the other *J. communis* varieties from North America. The only other member of section *Juniperus* in North America is *J. jackii* that is in a clade with *J. mairei* from China (Fig. 3).

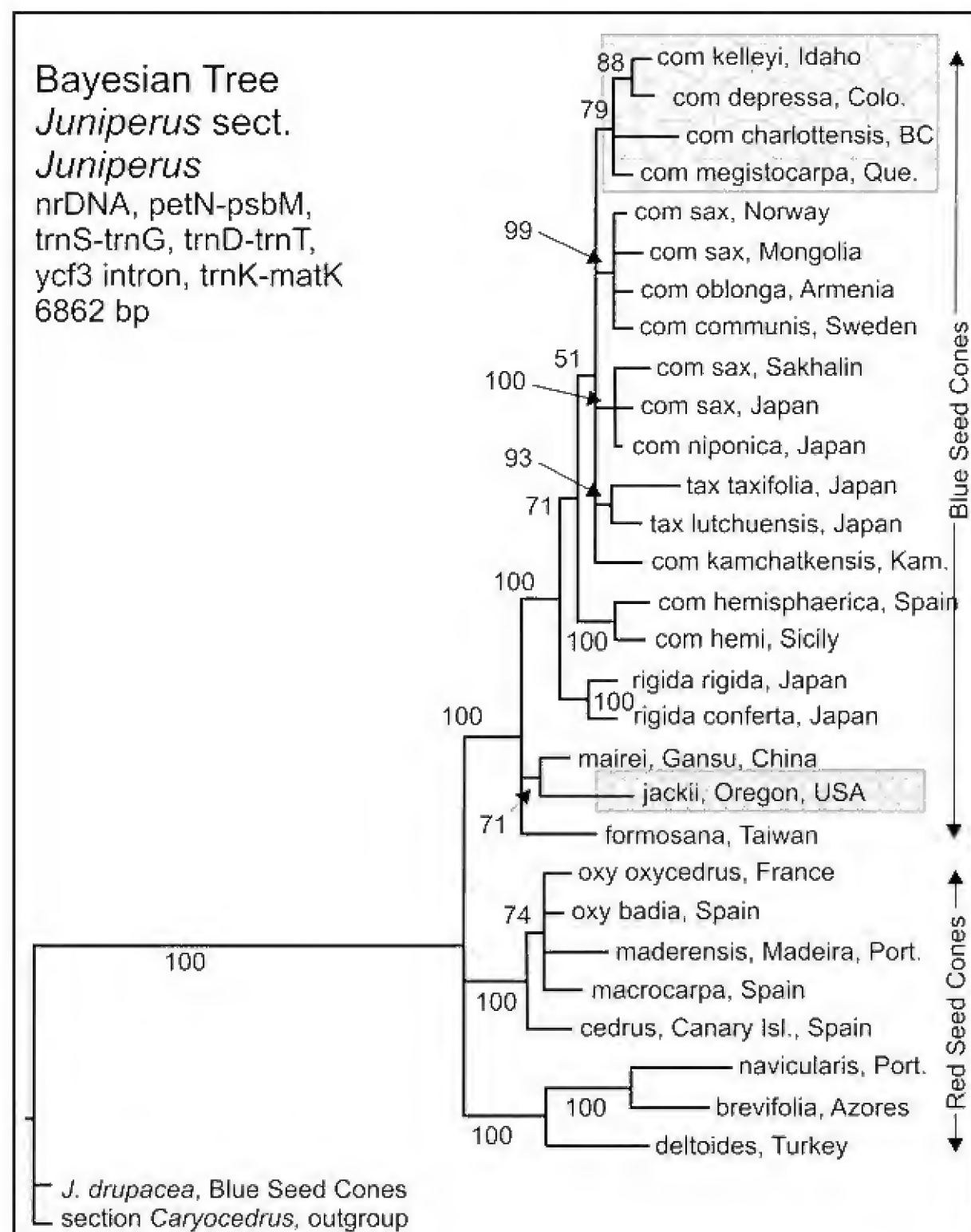


Figure 3. Bayesian tree for all taxa of *Juniperus* sect. *Juniperus* taxa. Numbers at the branch points are posterior probabilities (as percent). Adapted from Adams and Schwarzbach (2012).

A minimum spanning network of the taxa of section *Juniperus* shows that *J. communis* var. *kelleyi* differs by only 2 MEs (mutational events) from var. *depressa* (Fig. 4), but var. *kelleyi* is a number of MEs different from the *J. communis* complex in Europe and central Asia (Fig. 4).

A comparison of the leaf essential oil of var. *kelleyi* with var. *saxatilis* (Europe, Table 1) shows that these taxa differ in numerous components: α -pinene, sabinene, β -pinene, δ -3-carene, limonene, β -phellandrene, γ -terpinene, cis-sabinene hydrate, trans-sabinene hydrate, trans-thujone, terpinen-4-ol, myrtenol, citronellol, bornyl acetate, citronellyl acetate, neryl acetate, geranyl acetate, α -bisabolol, shyobunol, and 4 diterpenes. In fact, var. *saxatilis* (Europe, Table 1) seems to share a greater similarity in its oils with *J. jackii*, than with var. *kelleyi* (Table 1). The leaf oil of var. *kelleyi* is dominated by α -pinene (56.5%), δ -3-carene (11.5%) and β -pinene (5.4%) and is very similar to the oil of var. *depressa* (a similarity also seen in DNA sequence data, Figs. 3, 4).

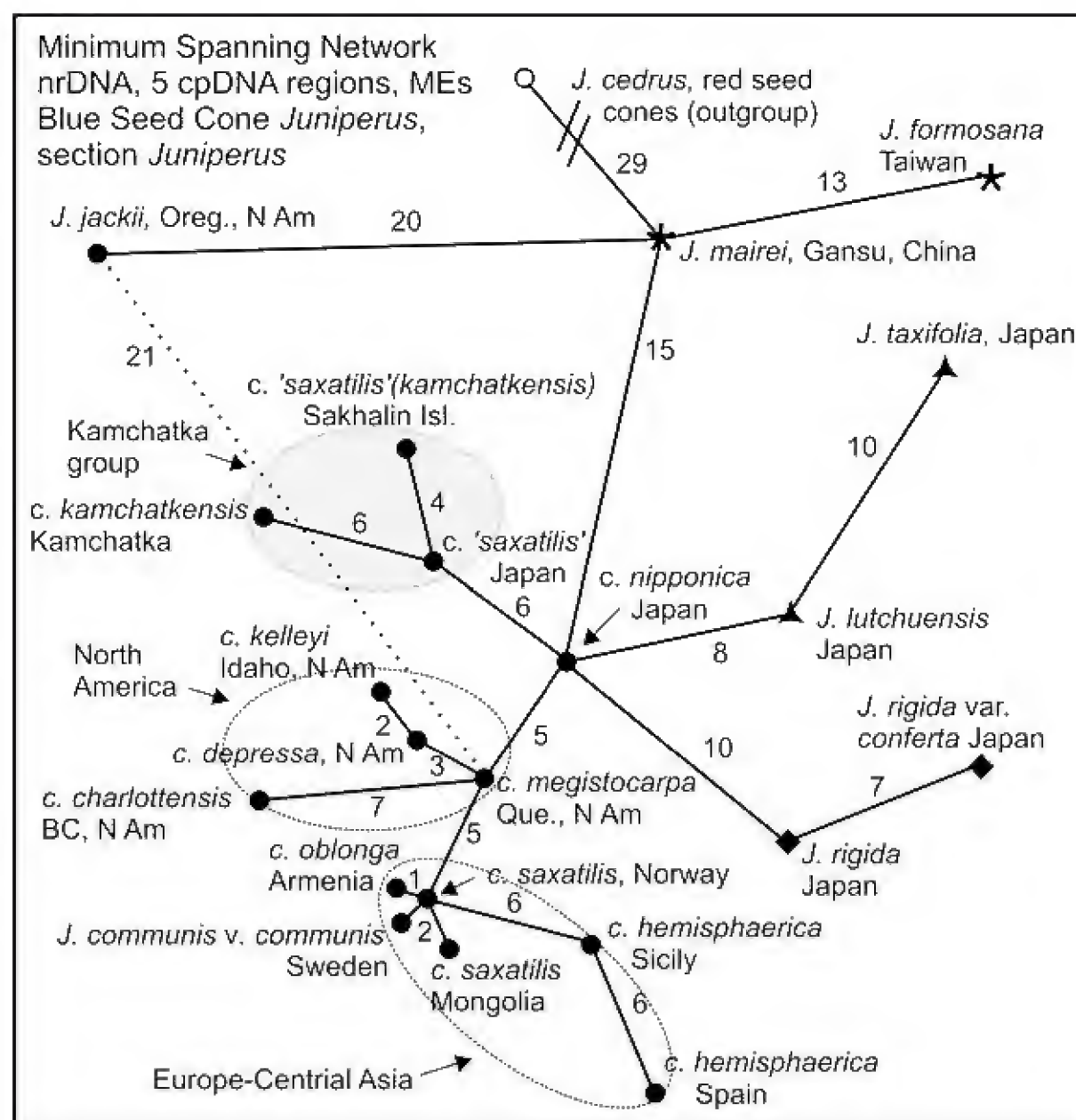


Figure 4. Minimum spanning network (MSN) of the blue seed cone junipers (see notes Fig. 4). *J. cedrus* is the nearest of the red seed cone species. Note that *c. var. kelleyi*, Idaho, North America is separated by only 2 MEs from *c. depressa* and is not in the group with *c. saxatilis* from Europe - Central Asia. Adapted from Adams and Schwarzbach (2012).

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Table 1. Comparison of the leaf oils of *J. communis* var. *kelleyi* with other varieties from North America and Europe and *J. jackii*. Taxa: *J. jackii* from serpentine, northwest California (*jackii*¹) and lava, Mt Hood, n Oregon (*jackii*²); var. *charlottensis*, Queen Charlotte Island, BC (*char*); var. *depressa*, Guadalupita, NM (*dep*); var. *megistocarpa*, Magdalen Islands, Quebec (*meg*); var. *kelleyi* (Little Redfish Lake, ID); var. *saxatilis*, Switzerland, Europe (*sax Eu*); and var. *communis*, Stockholm, Sweden (*com Sw*). Data from Adams et al. (2010) and Adams (2000). Compounds in bold face appear to separate taxa.

KI	Compound	North America						Europe	
		<i>jackii</i> ¹	<i>jackii</i> ²	<i>char</i>	<i>meg</i>	<i>dep</i>	<i>kelleyi</i>	<i>sax Eu</i>	<i>com Sw</i>
854	(E)-2-hexenal	0.2	1.0	0.4	0.1	0.1	0.3	1.2	0.7
926	tricyclene	t	t	0.1	0.1	0.1	0.1	t	0.3
931	α -thujene	t	t	t	t	t	t	4.1	0.1
939	α-pinene	16.1	18.9	59.3	58.5	53.9	56.5	14.1	56.8
953	α -fenchene	0.3	0.6	0.1	t	1.0	0.5	0.1	0.3
953	camphene	0.3	0.6	0.6	0.6	1.0	0.5	0.2	0.6
954	thuja-2,4-diene	-	-	0.1	-	0.1	-	-	-
967	verbenene	0.3	0.3	-	-	-	-	-	-
976	sabinene	0.1	0.3	0.3	0.3	0.3	0.3	32.8	0.7
978	1-octen-3-ol	0.1	-	-	t	t	-	-	-
980	β-pinene	1.9	1.9	5.9	5.0	5.5	5.4	1.9	4.4
991	myrcene	3.2	3.2	4.8	3.9	4.1	4.5	5.0	5.2
997	ethyl hexanoate	-	-	0.1	-	-	-	-	-
1001	δ -2-carene	0.2	0.2	0.1	0.2	0.2	0.1	0.4	0.2
1005	α -phellandrene	2.2	2.5	0.1	0.1	0.2	0.1	0.5	2.1
1011	δ-3-carene	17.9	28.4	3.6	0.7	9.3	11.5	0.5	4.7
1018	α -terpinene	-	-	-	0.1	t	-	1.9	t
1026	p-cymene	1.1	0.8	0.2	0.1	0.2	t	0.3	0.3
1031	limonene	6.6	0.5	1.9	20.4	2.6	2.1	6.7	5.1
1031	β-phellandrene	13.4	9.2	2.9	1.0	2.5	3.1	0.6	8.9
1050	(E)- β -ocimene	0.3	t	-	t	t	-	0.1	0.1
1057	amyl isobutyrate	-	-	-	-	-	-	-	0.2
1062	γ-terpinene	0.1	0.1	0.1	0.1	0.1	0.1	3.4	t
1068	cis-sabinene hydrate	-	-	-	t	-	t	1.8	t
1088	terpinolene	3.2	4.4	1.0	0.5	1.4	1.8	3.0	1.1
1095	linalool	-	-	0.1	0.4	0.3	0.2	-	0.1
1097	trans-sabinene hydrate	-	-	-	-	-	-	1.3	-
1100	n-nonanal	-	-	0.1	-	-	-	-	-
1103	isoamyl-isovalerate	-	-	-	-	-	-	t	0.1
1112	3-methyl-3-butenyl-								
	isovalerate	-	-	t	t	0.1	0.1	-	t
1114	trans-thujone (= β -thujone)	-	-	-	-	-	-	0.6	-
1121	cis-p-menth-2-en-1-ol	0.2	0.1	-	t	-	t	-	t
1125	α -campholenal	0.2	0.2	0.4	t	0.5	0.1	-	t
1132	cis-limonene oxide	0.1	0.4	-	0.2	0.2	-	-	-
1133	cis-p-mentha-2,8-dien-1-ol	-	-	-	-	-	0.1	-	-
1139	trans-pinocarveol	0.2	0.3	0.4	t	0.5	0.1	-	-
1141	camphor	-	-	0.2	-	-	0.2	-	-
1143	trans-verbenol	0.2	0.3	0.2	t	0.7	-	-	-
1147	3-methyl-2-butenyl-								
	isovalerate	-	-	-	-	0.1	0.2	-	t
1148	citronellal	-	-	-	0.2	0.2	0.1	-	-
1158	pinocarvone	-	-	0.1	-	-	-	-	-
1159	p-mentha-1,5-dien-8-ol	0.4	0.3	0.3	-	0.5	0.1	-	t
1165	borneol	-	-	0.3	0.2	-	0.1	t	0.2
1172	cis-pinocamphone	-	-	0.1	-	-	0.1	-	-
1177	terpinen-4-ol	0.7	0.3	0.5	0.2	0.5	0.3	7.3	0.2
1176	m-cymen-8-ol	-	-	0.1	-	0.2	-	-	-
1179	naphthalene	-	-	0.1	0.2	-	-	0.3	t

KI	Compound	<i>jackii</i> ¹	<i>jackii</i> ²	<i>char</i>	<i>meg</i>	<i>dep</i>	<i>kelleyi</i>	<i>sax</i> Eu	<i>com</i> Sv
1183	p-cymen-8-ol	0.3	0.3	0.1	-	0.2	-	t	t
1189	α -terpineol	0.3	0.3	1.0	1.5	0.6	0.5	0.4	0.2
1190	methyl salicylate	-	-	0.1	-	-	0.1	-	-
1194	myrtenol	0.4	0.3	0.3	0.5	0.5	0.3	-	-
1204	verbenone	0.3	0.5	0.3	-	0.3	t	-	t
1217	trans-carveol	0.4	t	0.3	t	0.2	t	-	-
1223	citronellool	-	-	0.1	0.3	0.5	0.2	-	-
1235	methyl thymol	0.2	0.2	-	t	-	0.1	0.1	-
1239	carvone	-	-	0.1	-	-	0.1	-	-
1249	piperitone	-	-	-	t	0.4	0.2	-	t
1257	methyl citronellate	-	-	0.2	t	0.1	0.3	-	t
1285	bornyl acetate	0.5	0.5	1.0	0.5	0.6	0.7	0.2	0.9
1291	trans-verbenyl acetate	-	-	-	t	-	-	-	-
1292	(E,Z)-2,4-decadienal	-	-	t	t	-	t	-	-
1293	methyl myrtenate	0.2	0.5	-	-	-	-	-	-
1302	α-terpinyl formate	1.0	1.5	1.0	-	0.2	0.3	-	-
1312	citronellic acid	-	-	-	t	t	t	-	-
1324	myrtenyl acetate	1.6	2.7	1.2	1.1	1.1	1.0	-	t
1332	cis-piperitol acetate	-	-	-	-	-	0.1	-	-
1365	cis-carvyl acetate	-	-	0.1	-	-	t	-	-
1350	α -terpinyl acetate	0.9	5.8	t	0.2	1.7	0.6	0.5	-
1350	citronellyl acetate	-	-	0.1	t	0.3	0.1	-	t
1359	neryl acetate	-	-	0.1	t	0.1	0.1	-	-
1379	geranyl acetate	-	-	0.1	0.1	1.3	0.3	-	-
1381	trans-myrtanyl acetate	t	t	-	-	-	-	t	-
1391	β -elemene	0.3	0.1	0.2	t	0.1	0.1	t	0.2
1418	(E)-caryophyllene	0.4	t	t	-	-	0.1	t	0.7
1448	cis-muurolo-3,5-diene	-	-	0.1	-	-	-	-	-
1454	α -humulene	0.5	0.2	0.1	-	t	0.1	t	0.5
1465	cis-muurolo-4(14),5-diene	t	t	0.1	-	-	-	-	-
1475	trans-cadina-1(6),4-diene	-	-	0.1	-	-	0.1	-	-
1477	γ -muurolene	t	t	0.1	t	0.1	0.1	t	t
1480	germacrene D	4.1	1.1	0.3	0.1	0.2	0.6	0.4	0.7
1493	trans-muurolo-4(14),5-dier	-	-	0.1	-	-	0.1	-	-
1493	epi-cubebol	0.3	t	0.2	-	-	-	-	t
1499	α -muurolene	0.6	0.2	0.3	0.1	0.1	0.2	0.2	0.2
1503	germacrene A	t	t	0.1	t	0.1	0.1	0.2	0.1
1505	β-bisabolene	-	-	0.1	-	-	-	-	-
1513	γ -cadinene	1.2	0.4	0.4	0.1	0.2	0.3	0.4	0.2
1524	δ -cadinene	2.2	0.7	1.4	0.4	0.5	0.7	0.8	0.5
1538	α -cadinene	0.2	0.1	0.1	t	0.1	0.1	t	t
1549	elemol	t	-	-	-	t	t	-	t
1556	germacrene B	0.5	0.3	0.3	-	0.3	1.2	0.3	0.3
1561	(E)-nerolidol	-	-	t	0.1	t	t	-	-
1574	germacrene D-4-ol	0.9	0.8	0.8	0.5	1.0	1.2	1.8	0.8
1577	spathulenol	-	-	0.1	0.1	-	0.1	-	t
1607	β -oplophenone	-	-	0.1	t	-	0.1	-	-
1581	caryophyllene oxide	0.2	t	-	-	-	-	-	t
1594	salvial-4(14)-en-1-one	0.1	-	-	-	-	-	-	-
1606	humulene epoxide II	t	t	-	-	0.1	-	-	t
1627	1-epi-cubenol	1.5	t	t	-	-	t	t	t
1640	epi- α -cadinol	0.7	0.3	0.3	0.1	0.2	0.2	0.5	t
1640	epi- α -muurolol	0.8	0.3	0.3	0.1	0.2	0.3	0.5	0.4
1645	α -muurolol	0.4	0.1	0.1	t	0.1	0.1	0.1	t
1653	α -cadinol	2.0	1.1	0.9	0.3	0.7	0.8	1.3	0.5
1685	α-bisabolol	-	-	1.0	t	0.7	0.2	-	-
1685	germacra-4(15),5,10(14)-trien-1-al	0.3	-	-	-	-	-	-	t
1688	shyobunol	t	t	0.2	-	0.1	0.3	-	0.7

KI	Compound	<i>jackii</i> ¹	<i>jackii</i> ²	<i>char</i>	<i>meg</i>	<i>dep</i>	<i>kelleyi</i>	<i>sax</i> Eu	<i>com</i> Sv
1714	(2E,6Z)-farnesal	-	-	-	t	-	-	-	-
1722	(2Z,6E)-farnesal	-	-	-	0.3	-	-	-	-
1742	(2E,6E)-farnesal	-	-	-	t	-	-	-	-
1806	nootkatone	-	-	0.1	-	-	-	-	-
1933	cyclohexadecanolide	0.1	-	0.1	-	-	-	-	-
1968	sandaracopimara-8(14), 15-diene	-	t	-	-	-	-	-	-
2022	abieta-8,12-diene	-	t	-	-	-	-	-	-
1989	manoyl oxide	0.2	0.3	-	-	-	-	0.1	-
2055	abietatriene	0.3	0.6	0.1	-	-	-	0.2	-
2056	manool	0.6	0.7	-	-	-	-	-	-
2080	abietadiene	-	1.1	-	-	-	-	0.4	-
2106	isoabienol	0.2	0.9	-	-	-	-	0.1	-
2331	trans-ferruginol	t	0.2	-	-	-	-	-	-

KI = Kovat's Index on DB-5(= SE54) column. *Tentatively identified. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Taxonomy of *Tecoma stans* (Bignoniaceae) in North America

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ABSTRACT

Tecoma stans in North America is treated as having two morphological meaningful varieties: a typical, widespread, highly variable var. **stans** and a regionally, much more uniform, var. **angustata**. The, often recognized, var. *velutina*, is reduced in rank to **forma velutina** (DC.) B.L. Turner, **stat. nov.** A key to the several taxa is provided, along with maps showing their distributions. Published on-line www.phytologia.org *Phytologia* 95(2): 222-225 (August 1, 2013).

KEY WORDS: Bignoniaceae, *Tecoma*, *T. angustata*, *T. stans*, Mexico

Preoccupation with the *Tecoma* complex in Mexico has occasioned the present paper.

Shinners (1961) provided a scholarly account of *Tecoma*, noting its establishment and that it is typified by **T. stans** (L.) Juss. ex H.B.K., presumably from pre-Linnean collections assembled in the West Indies; he stated further:

It is native in the West Indies, Mexico, and Central America, showing considerable variation as to form, tothing, and pubescence of leaflets. A fairly uniform race native in extreme western Texas and northern Mexico, with narrow, deeply cut, glabrous leaflets is *T. stans* var. *angustata* Rehder, Mitt. Deutsch. Dendrol. Ges. 1915: 227, the type (not seen; presumably at the Arnold Arboretum) from El Paso, Texas (M. E. Jones 4187, Sept. 10, 1883).

Shinners further noted that var. **angustata** had been treated as a distinct species by various workers, but concluded that such recognition was unjustified, but he accepted its infraspecific rank. Standley (1961), however, disagreed, stating that:

The species is a variable one, but although the writer has spent a large amount of time in study of the extensive series of specimens available, it has been impossible to find any reliable characters by which to assign the material to one or more species. In the typical form the leaflets are usually glabrous. *T. stans velutina* is a form in which the leaflets are pubescent or tomentose beneath. This has usually been maintained as a distinct species, but there is every possible graduation between the forms with glabrous leaflet and those with tomentose ones. *Tecoma stans angustata* Rehder (*Stenolobium incisum* Rose & Standl.) is a form common in northeastern Mexico and the adjacent United States in which the leaves are usually narrow and incised-serrate.

Gentry (1982), in his account of *Tecoma* for the state of Veracruz, treated *T. s.* var. **angustata** as synonymous with var. **stans**, but recognized *T. s.* var. *velutina* as distinct, noting it to be similar to var. **stans**, but possessing very pubescent foliage and occurring at somewhat higher elevations.

My own studies and observations, both in the field and in herbaria, lead me to believe that there are only two meaningful populational categories in north America: var. **angustata** and var. **stans**, the latter possessing forms with markedly pubescent foliage, these referred to as var. *velutina* by most authors, as noted in the above paragraph; plants of the latter occur sporadically throughout most of the range of var. **stans** and are reduced to the status of forma in the present account.

Key to North American taxa of *Tecoma stans*

1. Leaflets mostly linear-lanceolate, 0.8-1.5 cm wide, glabrous, having margins sharply serrate, their apices sharply acute; north-central Mexico and closely adjacent USA.....var. **angustata**
1. Leaflets more nearly ovate to lanceolate, 1.0-4.0 cm wide, to some degree pubescent (rarely not), margins not sharply serrate, their apices not sharply acute...(2)
2. Leaflets nearly glabrous to sparsely pubescent.....var. **stans**
2. Leaflets markedly pubescent.....forma **velutina**

TECOMA STANS (L.) Juss. ex H.B.K., Nov. Gen. 3: 144. 1818.

Tecoma stans* var. *stans

Begonia stans L.

Gelsemium stans (L.) Kuntze

Stenolobium stans (L.) Seem.

S. stans var. *pinnata* Seem.

Tecoma stans var. *apiifolium* Hort. ex DC.

[Gentry (1982) provides a more extended synonymy]

This, the typical variety, occurs throughout most of central and southern Mexico southwards to South America and is readily recognized, both in the field and in herbaria. It is exceedingly variable, as declared by most workers, as noted above, and by both Gentry (1992) and Wood (2008), who recognized at least two of the varietal taxa in South America [var. **stans** and var. *velutina* (f. *velutina*, of the present author)].

Gentry (1982) provided an excellent line sketch of the typical variety.

Tecoma stans* var. *angustata Rehder, Mitt. Deutsch. Dendrol. Ges. 1915: 227.

Stenolobium incisum Rose & Standl.

This is a well-marked variety, forming large, relatively uniform populations over a large area. It is essentially allopatric with var. **stans**, but occasional weakly intergrading plants can be found where their ranges approach each other, as exemplified by the following collections: **Mexico. Nuevo Leon:** 11 mi S of St. Catarina, on “almost perpendicular rock walls.” *Carlson* 2694 (TEX); **Mpio. Zaragoza** (23 58 N x 99 48 W), *Patterson* 5962 (TEX).

Interestingly, Gentry (1982) placed this name in synonymy under var. **stans**, but maintained var. *velutina*, which is clearly but a sporadic pubescent form of var. **stans**, as noted below; he subsequently (Gentry 1992) accepted the taxon but noted “This narrow-leafleted form intergrades with typical **T. stans** in central Mexico and perhaps does not even merit varietal status.” I strongly disagree with his latter assessment and have little doubt as to its geomorphological integrity.

Benson (1944) provided an excellent line drawing of the taxon and Epple and Epple (1995) provided a photo of the living plant.

***Tecoma stans* forma *velutina* (DC.) B.L. Turner, stat. nov.**

Based upon *Tecoma stans* var. *velutina* DC., Prodr. 9: 224. 1845.

Gelsemium molle (H.B.K.) Kuntze

Stenolobium molle (H.B.K.) Seem.

Tecoma mollis H.B.K.

This form is found sporadically throughout the range of var. **stans**, usually at somewhat higher elevations, as noted by Gentry (1982); it often occurs with the latter and numerous intermediates can be found where they coexist, nothing but pubescence separating the two. In short, they are not geomorphologically coherent, nor do they form meaningful populational aggregates, hence their treatment as but forms, such individuals hop-scotching from Mexico through Central America into South America. Rzedowski and Rzedowski (1993) voice similar sentiments regarding the status of var. *velutina* in their Flora del Bajío, as does Seibert (1940) in his treatment of the taxon in Central America.

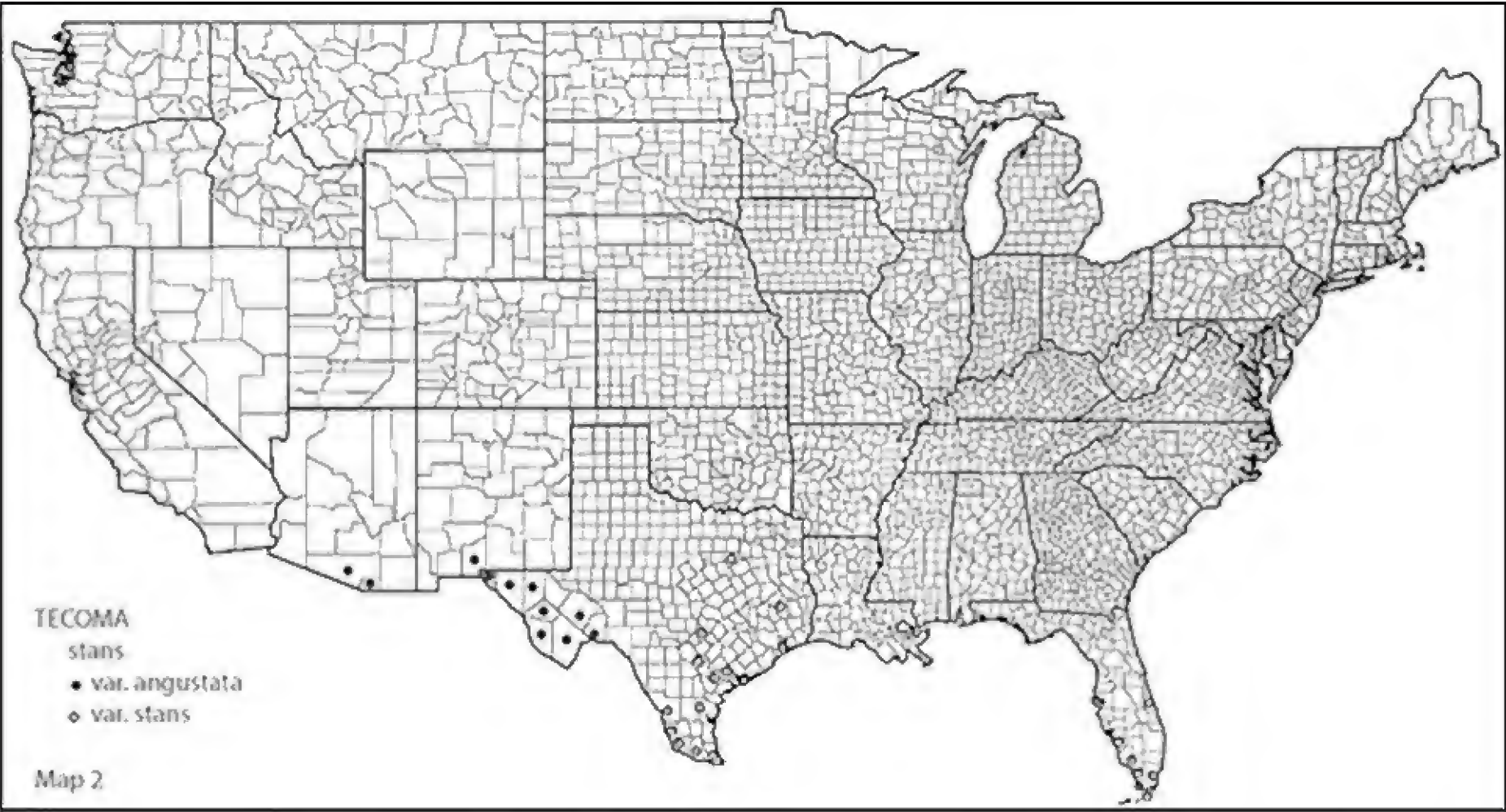
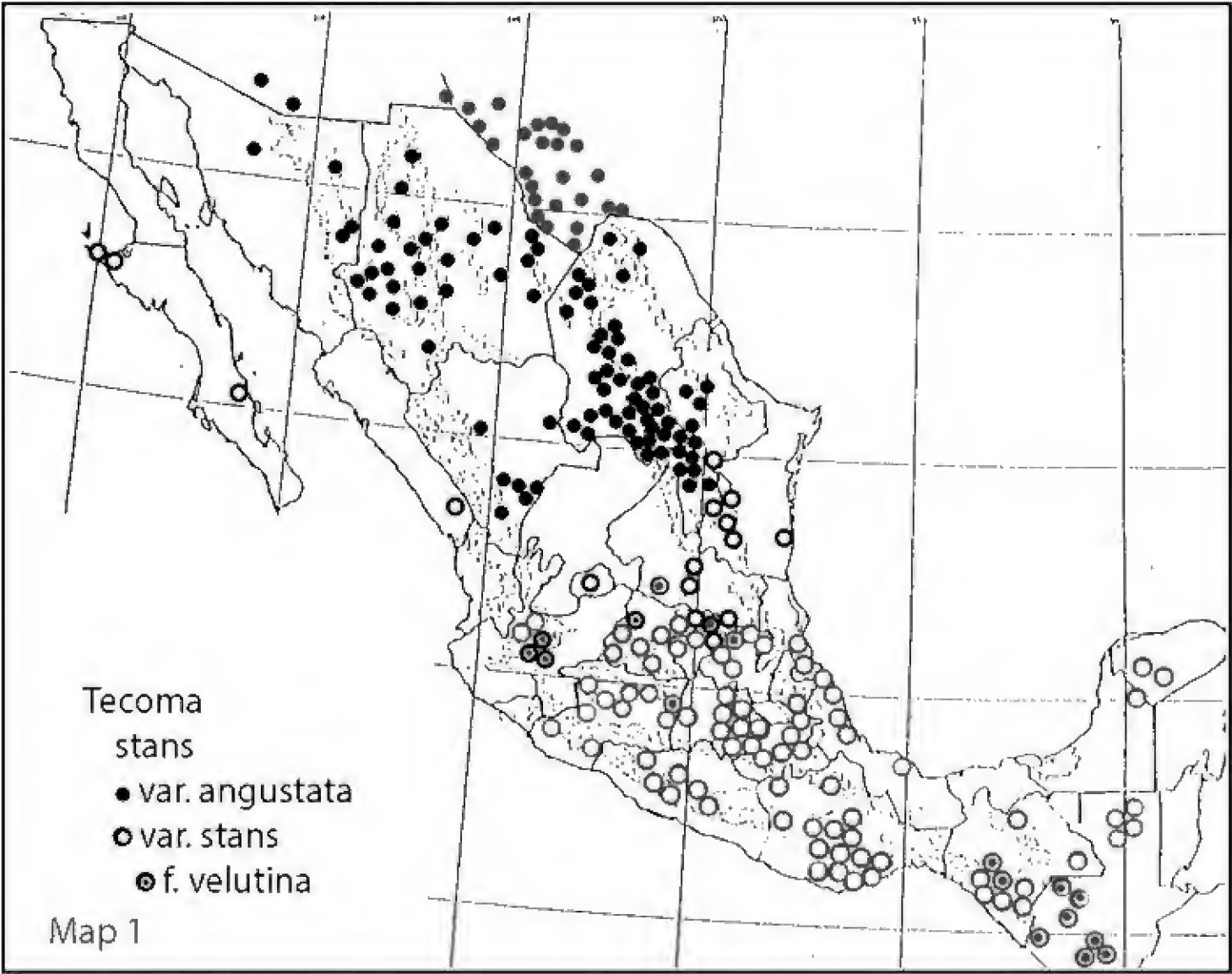
Distributions of the several taxa in Mexico are shown in Map 1; their distribution in the USA is shown in Map 2. All collections of **T. stans** var. **stans** in the USA are believed to have been introduced; it is also widely introduced in South America, Asia and Africa.

ACKNOWLEDGEMENTS

I am grateful to my close colleague, Jana Kos, for editorial assistance. The distribution maps are based upon specimens on file at LL-TEX, various floral treatments of Mexico, and USDA records available on the web.

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CORRECTION

The following paper:

R. P. Adams and A. E. Schwarzbach. 2012. Taxonomy of the multi-seeded, entire leaf taxa of the *Juniperus*, section *Sabina*: sequence analysis of nrDNA and four cp DNA regions. *Phytologia* 94(3): 350-366.

contained an error in figure 3. The corrected Fig. 3 shows *J. chinensis* var. *sargentii* linked to *J. chinensis* var. *procumbens* with a difference of 11 Mutational Events (MEs).

This correction in the nearest link of *J. c.* var. *sargentii* to *J. chinensis*, resulted in a change in the level of support for var. *sargentii* that is reflected in the revised Table 1 below.

Correction published on-line www.phytologia.org
Phytologia 95(3): 226- 227 (August 1, 2013).

KEY WORDS: *Juniperus*, sect. *sabina*, correction.

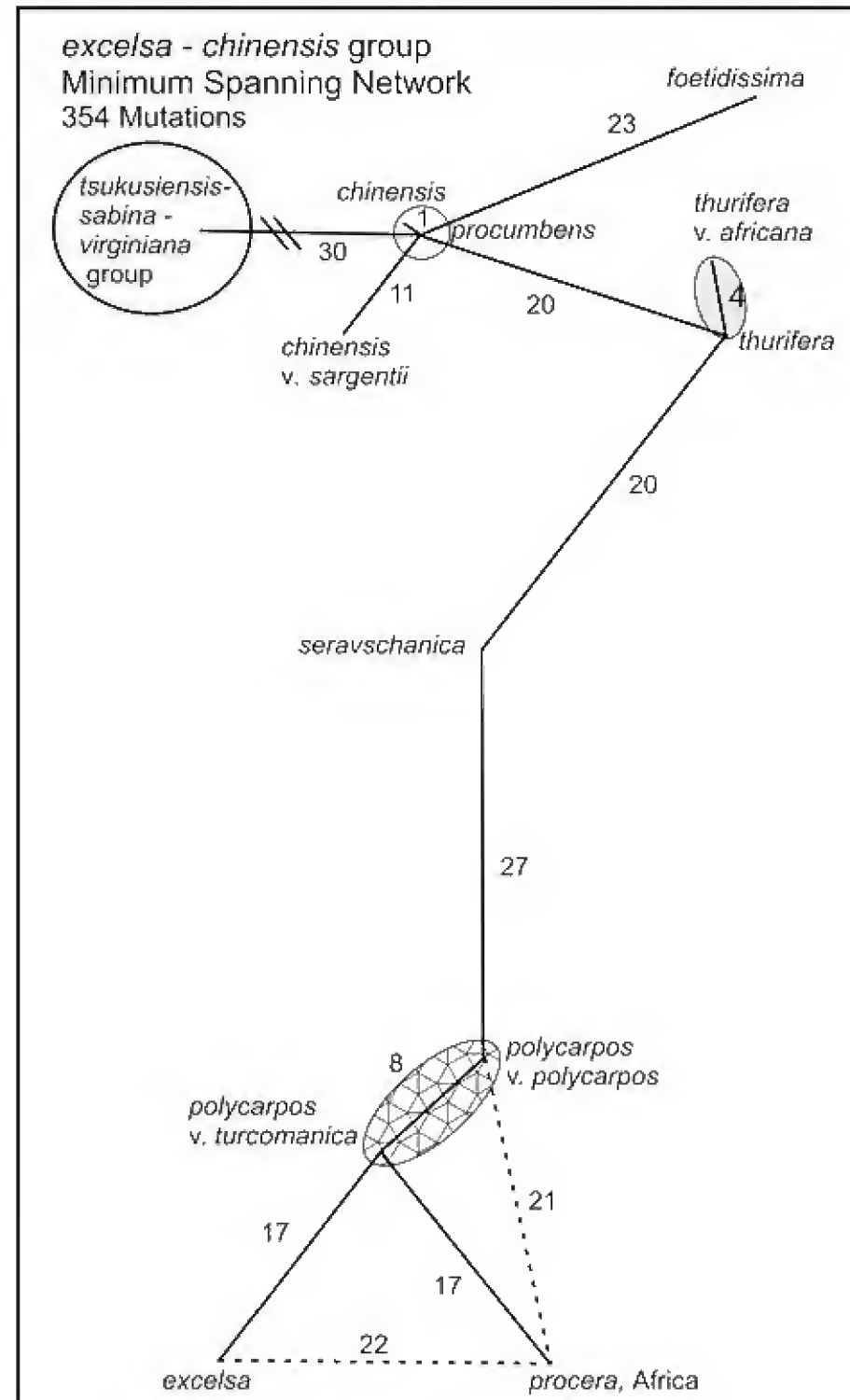


Figure 3 (revised). Minimum spanning network of the *excelsa*-*chinensis* group. Numbers next to links are the number of Mutational Events (MEs). Dashed lines are the second nearest links.

Table 1 (revised). Comparison of support using DNA sequencing data versus the taxonomies of Adams (2011) and Farjon (2010). Support levels: ++ = very strong, + = strong, +/- = not strong, - = not supported.

Adams(2011)	Farjon (2005, 2010)	Supported by DNA data
<i>J. barbadensis</i>	<i>J. barbadensis</i>	++ <i>J. barbadensis</i>
<i>J. b. v. lucayana</i>	<i>J. b. v. lucayana</i>	+ <i>J. barbadensis</i> or <i>J. b. v. lucayana</i>
<i>J. bermudiana</i>	<i>J. bermudiana</i>	++ <i>J. bermudiana</i>
<i>J. blancoi</i>	<i>J. blancoi</i>	++ <i>J. blancoi</i>
<i>J. b. v. huebuentensis</i>	<i>J. blancoi</i>	++ <i>J. b. v. huebuentensis</i>
<i>J. b. v. mucronata</i>	<i>J. b. v. mucronata</i>	++ <i>J. b. v. mucronata</i>
<i>J. chinensis</i>	<i>J. chinensis</i>	++ <i>J. chinensis</i>
<i>J. c. v. sargentii</i>	<i>J. c. v. sargentii</i>	+ <i>J. c. v. sargentii</i>
<i>J. c. v. tsukusiensis</i>	<i>J. c. v. tsukusiensis</i>	++ <i>J. tsukusiensis</i>
<i>J. c. v. taiwanensis</i>	<i>J. c. v. tsukusiensis</i>	++ <i>J. t. v. taiwanensis</i>
<i>J. erectopatens</i>	<i>J. chinensis</i>	++ <i>J. erectopatens</i>
<i>J. excelsa</i>	<i>J. excelsa</i> (in part)	++ <i>J. excelsa</i>
<i>J. foetidissima</i>	<i>J. foetidissima</i>	++ <i>J. foetidissima</i>
<i>J. gracilior</i>	<i>J. gracilior</i>	++ <i>J. gracilior</i>
<i>J. g. v. ekmanii</i>	<i>J. g. v. ekmanii</i>	++ <i>J. g. v. ekmanii</i>
<i>J. g. v. urbaniana</i>	<i>J. g. v. urbaniana</i>	++ <i>J. g. v. urbaniana</i>
<i>J. horizontalis</i>	<i>J. horizontalis</i>	++ <i>J. horizontalis</i>
<i>J. jarkendensis</i>	<i>J. semiglobosa</i>	++ <i>J. s. v. jarkendensis</i>
<i>J. maritima</i>	<i>J. scopulorum</i>	++ <i>J. maritima</i>
<i>J. phoenicea</i>	<i>J. phoenicea</i>	++ <i>J. phoenicea</i>
<i>J. p. v. turbinata</i>	<i>J. p. subsp. turbinata</i>	++ <i>J. turbinata</i>
<i>J. procera</i>	<i>J. procera</i>	++ <i>J. procera</i>
<i>J. procumbens</i>	<i>J. procumbens</i>	++ <i>J. chinensis v. procumbens</i>
<i>J. polycarpus</i>	<i>J. excelsa subsp. polycarpus</i>	++ <i>J. polycarpus</i>
<i>J. p. v. seravschanica</i>	<i>J. e. subsp. polycarpus</i>	++ <i>J. seravschanica</i>
<i>J. p. v. turcomanica</i>	<i>J. e. subsp. polycarpus</i>	+ <i>J. p. v. turcomanica</i>
<i>J. sabina</i>	<i>J. sabina</i>	++ <i>J. sabina</i>
<i>J. s. v. arenaria</i>	<i>J. s. v. arenaria</i>	++ <i>J. davurica v. arenaria</i>
<i>J. s. v. davurica</i>	<i>J. s. v. davurica</i>	++ <i>J. davurica</i>
<i>J. s. v. mongolensis</i>	<i>J. s. v. arenaria</i>	++ <i>J. davurica v. mongolensis</i>
<i>J. scopulorum</i>	<i>J. scopulorum</i>	++ <i>J. scopulorum</i>
<i>J. semiglobosa</i>	<i>J. semiglobosa</i>	++ <i>J. semiglobosa</i>
<i>J. saxicola</i>	<i>J. saxicola</i>	++ <i>J. gracilior v. saxicola</i>
<i>J. thurifera</i>	<i>J. thurifera</i>	++ <i>J. thurifera</i>
<i>J. t. v. africana</i>	<i>J. thurifera</i>	+/- <i>J. thurifera v. africana</i>
<i>J. virginiana</i>	<i>J. virginiana</i>	++ <i>J. virginiana</i>
<i>J. v. v. silicicola</i>	<i>J. v. v. silicicola</i>	+ <i>J. v. v. silicicola</i>

Two new species of *Stevia* (Asteraceae: Eupatorieae) from Oaxaca, Mexico

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ABSTRACT

Two new taxa of *Stevia* are described from the state of Oaxaca, Mexico: ***Stevia miahuatlana*** B.L. Turner, **sp. nov.**, from the environs of Cerro Quiexobra, Distrito Miahuatlan; and ***Stevia serboana*** B.L. Turner, **sp. nov.**, from the Distrito Solo de Vega. Photographs of the holotypes are provided, along with maps showing their distribution. Published on-line www.phytologia.org *Phytologia* 95(3): 228-232 (August 1, 2013).

KEY WORDS: Asteraceae, Eupatorieae, Mexico, Oaxaca, Distrito Miahuatlan, Cerro Quiexobra, Distrito Solo de Vega

Preoccupation with *Stevia* for the Comps of Mexico (Turner, 1997) stimulated the following account.

STEVIA MIAHUATLANA B.L. Turner, **sp. nov.** Fig. 1.

Perennial herbs, 50-80 cm high, presumably rhizomatous. **Mid-stems**, purplish, crinkly glandular-pubescent, the vestiture ca 0.5 mm high. **Leaves** (mid-stem) 4-8 cm long, 2-3 cm wide; petioles 0.5-3.0 cm long, weakly winged; blades broadly ovate to sub-deltoid, the margins irregularly dentate. **Capitulescence**, a terminal congested array of heads 3-6 cm high, 2-4 cm across; ultimate peduncles 1-4 mm long, glandular-pubescent, subtended by linear to linear-lanceolate bracts, 1.0-1.5 cm long. **Involucral bracts** 7-8 mm long, mostly linear-lanceolate, weakly to densely glandular-pubescent, their apices acute. **Corollas** rose to pale lavender (dried), pubescent without, the 5 lobes relatively small, ca 1 mm long. **Achenes** ca 5 mm long, devoid of bristles, the body black, sparsely pubescent near apex; pappus a crown of lacerate scales ca 0.5 mm high.

TYPE: MEXICO. OAXACA: Distr. Miahuatlan, Mpio. Santo Domingo Ozolotepec. “Laviche camino a la Mojonera, bosque de pino. ladera.” 16 32 12.7 N. 96 20 23.5 W, ca 2861 m, 10/02/2010, Silvia H. Salas M. 7163 (Holotype, TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. OAXACA: Mpio. Santo Domingo Ozolotepec, 35 km NE of Miahuatlan, 5 km NE of Santo Domingo Ozolotepec, “Timberline vegetation in open glades along ridges and in mountain saddles.” Very common throughout timberline and in pine forests, 3650-3800 m, 10 Dec 1989, *McDonald* 2946 (TEX); Cerro Quiexobra, “Montane vegetation on road to summit of sierra, pine forest or chaparral. Common weed in Chaparral-madrone woodland 11 km from summit of Cerro Quiexobra on logging road to La Cienegilla, 3200-3500 m, 11 Dec 1989, *McDonald* 2956 (TEX).

I had previously identified the two McDonald collections, cited above, as ***S. incognito*** Grashoff, a widespread variable species (**Map 1**) to which it will key to or near in my treatment of *Stevia* for Mexico (Turner 1997). While it has achenes w/o bristles, as does the latter, it differs in having smaller heads with shorter involucral bracts.

Stevia miahuatlana is not to be confused with ***S. quiexobrana*** B.L. Turner, typified by material collected by Hinton et al. from near the top of Mount Quiexobra (3385 m) in fir and pine woodlands, this readily identified by its small, sessile leaves. Judging from the cited localities above, it appears that ***S. miahuatlana*** occurs at somewhat lower elevations. Intermediates between the two have not been noted.

STEVIA SERBOANA B.L. Turner, *sp. nov.* **Fig. 2.**

Perennial herbs ca 50 cm high. **Stems** (upper) pubescent with crinkly white hairs ca 1 mm high. **Leaves** opposite throughout, 3-4 cm long, 2-3 cm wide; petioles 0.5-1.5 cm long; blades broadly ovate to sub-cordate, tapering into the petioles; upper surfaces greenish, moderately pubescent, lower surfaces whitened with a dense vestiture of crinkly hairs. **Capitulescence** an aggregation of 3-30 sessile heads, these borne on primary peduncles 3-6 cm long, the ultimate peduncles 0-1 mm long. **Involucral bracts**, linear-lanceolate, ca 6 mm long, their apices obtuse, pubescent with short, glandular hairs. **Florets** ca 10 mm long; corollas reportedly lilac, or rose-colored, ca 6 mm long, glabrous, or nearly so, their lobes ca 1.5 mm long. **Achenes** linear, ca 4 mm long, sparsely pubescent apically; pappus of 4 purplish bristles ca 4 mm long, between these 2-4 scales ca 0.5 mm high.

TYPE: MEXICO. OAXACA: Distrito, Solo de Vega; Mpio. Santiago Textitlan, “Tierra Blanca,” pine forests, ca 2299 m, 16 45 09 N, 97 11 24.3 W, 22 Jan 2007, *Maria Ester Jacob Salinas* (MJS) 1493 (Holotype, TEX).

ADDITIONAL SPECIMEN EXAMINED: same area as Type, but “Arriba de rio Tronco.” pine-oak forests, ca 1874 m 16 41 59 N, 97 15 28.7 W, 30 Dec 2006, *Vasquez* (RTV) 1130 (TEX).

In my treatment of *Stevia* for Mexico (Turner 1997), largely because of its glandular hairs, this novelty will key to or near **S. triangularis** Grashoff, a relatively rare species of Guerrero having very unusual, triangular leaves. It might also be confused with **S. tomentosa** HBK, because of its bicolored leaves (cf. **Map 2**).

The species name derives from the anagram SERBO, the group that provided funds for the collections concerned.

ACKNOWLEDGEMENTS

My editorial assistant, Jana Kos, kindly edited the paper and provided helpful comments. SERBO, provided the specimens concerned, for which I am grateful.

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Turner, B.L. 1997. *Stevia*, in Comps of Mexico 1. Phytologia Memoirs 11: 170-197.



Fig. 1. *Stevia miahuatlana*, holotype (TEX).

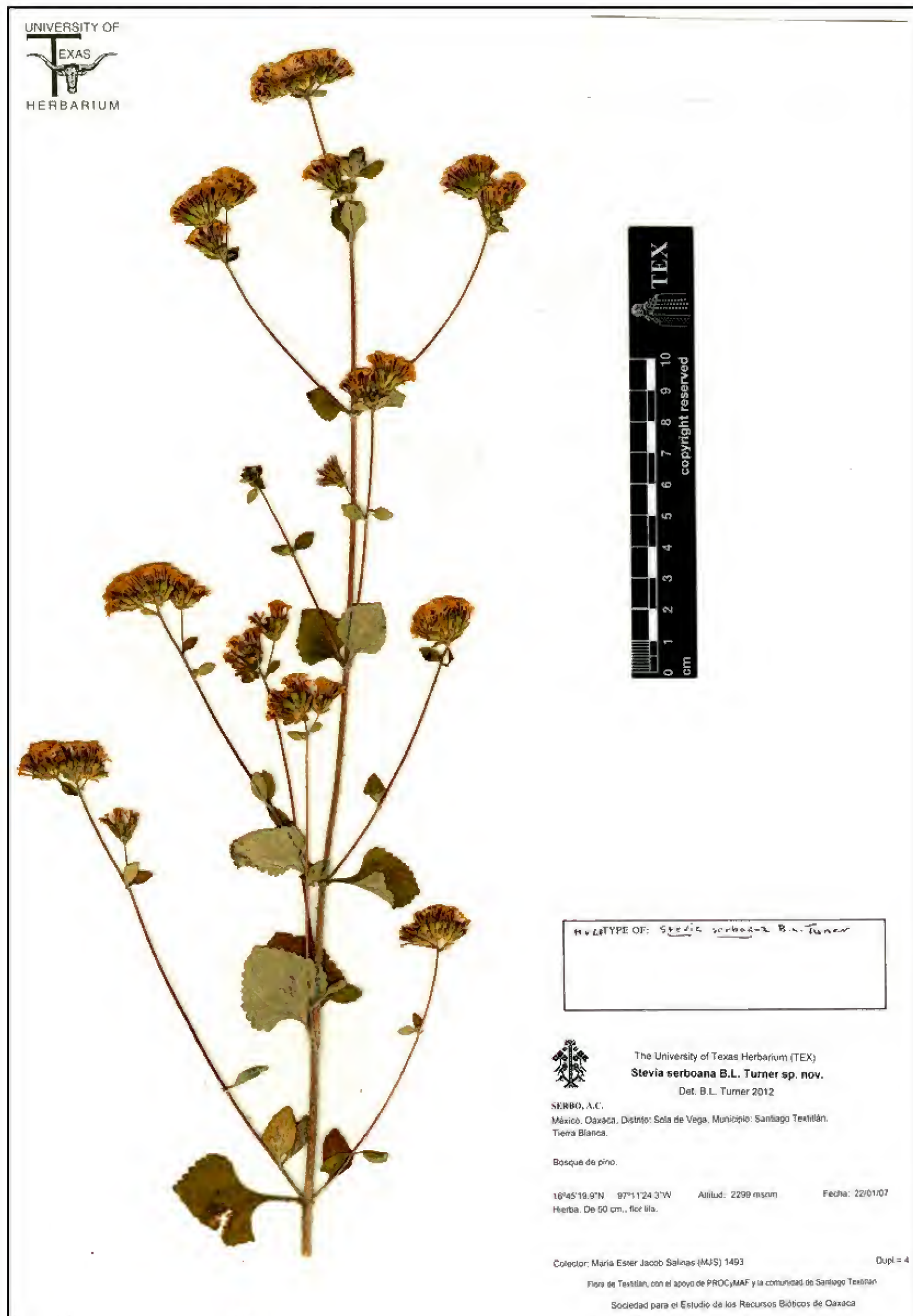
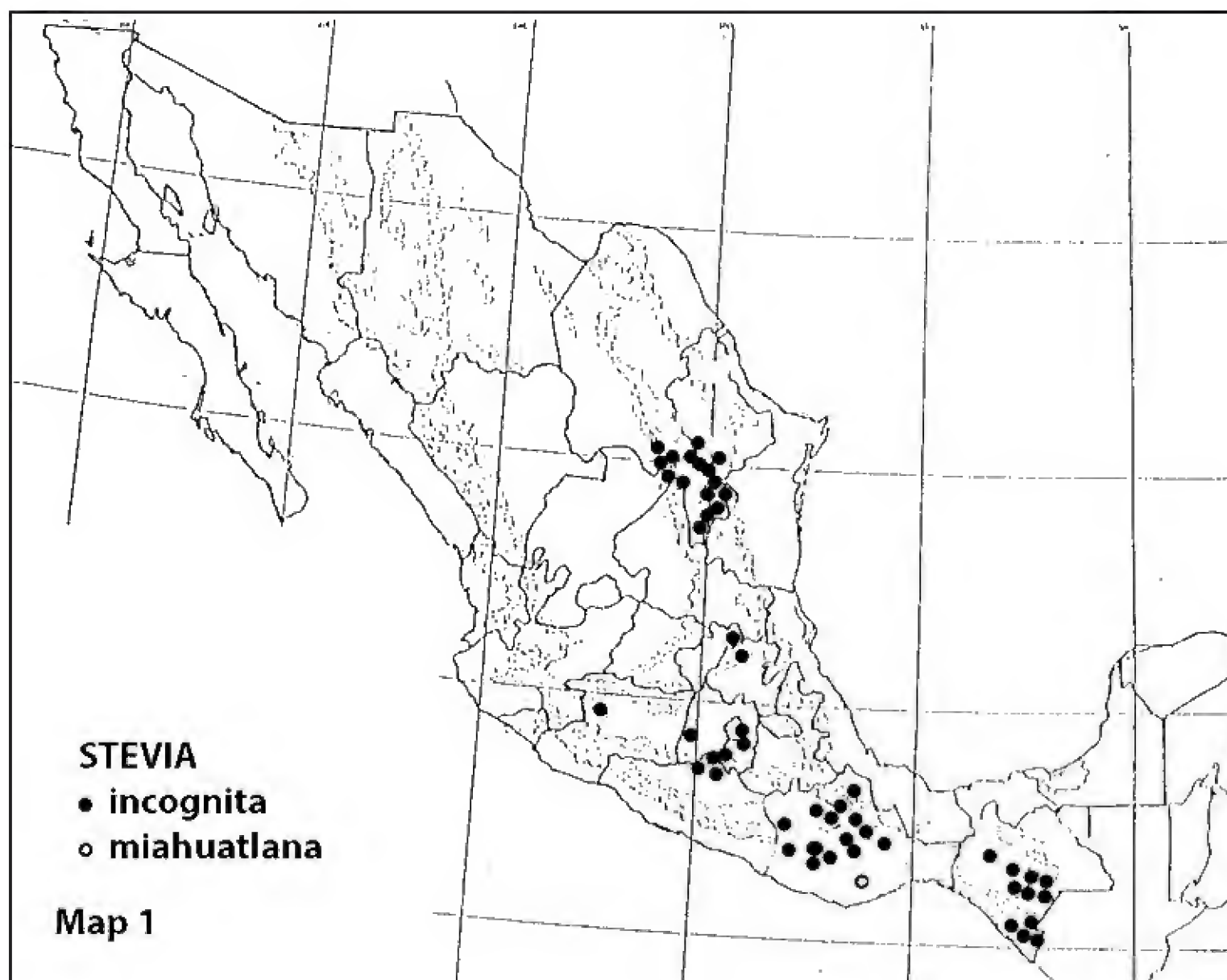
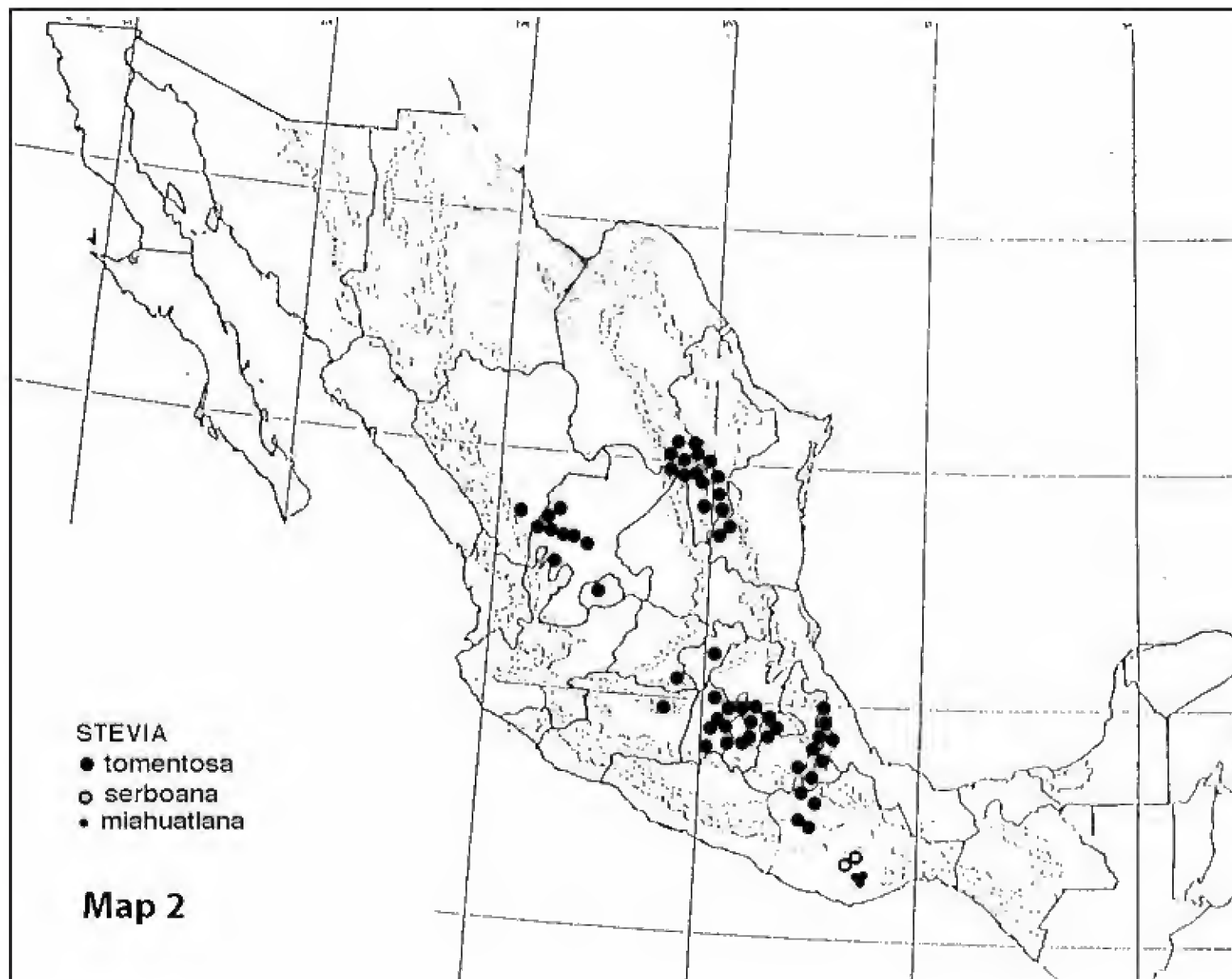


Fig. 2. *Stevia serboana*, holotype (TEX).



Map 1. Distribution of *Stevia incognita* and *S. miahutlana*.



Map 2. Distribution of *Stevia* spp.

Stevia reinana* (Asteraceae: Eupatorieae), a new species from near Yecora, Sonora, Mexico*Billie L. Turner**

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ABSTRACT

Stevia reinana B.L. Turner, **sp. nov.** is described from easternmost Sonora, Mexico. It is closely related to *S. glandulosa* but readily recognized by a syndrome of characters. A photograph of the type specimen is provided, along with maps showing the distribution of the taxa concerned. Published on-line www.phytologia.org *Phytologia* 95(3): 233-237 (August 1, 2013).

KEY WORDS: Asteraceae, Eupatorieae, *Stevia*, Mexico, Sonora

Stevia is a large, highly variable species complex, many of the taxa exhibiting varying degrees of polyploidy, this often complicated by asexual reproduction. In my treatment of the Mexican species (Turner 1997), I reckoned Mexico to harbor about 80 species; subsequent studies by Soejima et al. (2001) have raised the total to well over 90, and with the present contribution, and those of yet others, the number is now approaching 100.

STEVIA REINANA B.L. Turner, **sp. nov.** **Fig. 1**

Resembling *Stevia glandulosa* var. *gentryi* but the leaves weakly 3-nervate (vs strongly so), corollas white (vs pink), and 2 or 3 of their achenes having well developed bristles (vs squamellate); and involucre bracts 4-6 mm long (vs 7-8 mm).

Stiffly erect perennial herbs to 1 m high. Stems pilose with mostly recurved hairs. Leaves opposite, linear-lanceolate, 3-8 cm long, 0.4-1.0 cm wide, weakly 3-nervate and scarcely reticulately veined, the margins entire or nearly so; petioles indistinct, 0.1-3.0 mm long. Capitulescence a congested, terminal, corymbose panicle ca 3 cm across, the ultimate peduncles 0.5-3.0 cm long. Heads 7-8 mm high; involucre bracts 5-6 mm long, moderately pubescent with recurved hairs. Corollas white, ca 4 mm long, the lobes glabrous or nearly so, beset with a smattering of amber globules. Achenes, the body, glabrous or nearly so, ca 2.5 mm long; pappus of most achenes having elongate, well developed, bristles, the remaining having short, erose, squamellae.

TYPE: MEXICO. SONORA: Mpio. de Yécora, 3.2 km west of Restaurant Puerto de la Cruz, north slopes of Mesa del Campanero; pine-oak forest; 28 22 41 N, 109 02 40 W, 4 Jun 1999, *T. R. Van Devender* 99-240 [with A.L. Reina-G., P. West, K. Baker, B. Scarborough] (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. SONORA. Mpio. de Yécora, 3.5 km W of Arroyo Hondo, 9.7 km W of Chihuahua border on MEX 16, 28 26 06 N, 108 33 33 W, 1370 m, “mesic pine-oak forest on shady north slope,” 25 Sep 1997, *Reina G.* 97-1337; 4.8 km W of Puerto de la Cruz on MEX. 16, 1640 m, “shady sycamore canyon in pine-oak forest, 31 Aug 2000, *Reina-G.* 2000-556 (TEX); Restaurant La Palmita, N side of Mesa el Campanero, 1460 m, “oak woodland,” *Reina G.* 2000-799 (TEX); 2.7 mi W of Puerto de la Cruz, 1695 m, 22 Sep 1969, *Van Devender* 97-993 (TEX); 19 km SE of the junction with SON. 12 on MEX16, (near San Nicolás), 1420 m, “oak woodland,” *Van Devender* 98-2008 (TEX); 14.7 km E of Maycoba, 1460 m, “pine-oak forest,” *Van Devender* 99-630 (TEX); Mesa del Campanero, 2100 m, 1 Oct 2000, *Van Devender* 2000-830 (TEX).

Stevia reinana belongs to the Fruticosae grouping of *Stevia* (sensu Grashoff, 1972); it will key to or near *S. glandulosa* var. *gentryi* in my treatment of the Comps of Mexico (Turner 1997), differing in a number of characters, as noted in the above.

All of the specimens cited above are remarkably similar and presumably co-occur with *S. plummerae* (Figs. 2, 3) in a small area along MEX highway 16, just where it enters Sonora from Chihuahua. Nevertheless, putative hybrids or intermediates between these have not been detected among the numerous sheets of these taxa at LL-TEX, hence my treatment of the two taxa as distinct species.

The eponym honors Mrs. Ana Lilia Reina-Guerrero, loving mate of my long-time friend and colleague, Tom Van Devender, both having participated in the collection of type material. Long may their marriage endure!

Stevia reinana is partially sympatric with *S. glandulosa* var. *gentryi* (Figs. 2, 3) and occurs with, or near the latter in the Mpio. de Yécora, Sonora, as noted by the following collections:

S. glandulosa var. **gentryi**: Mpio. de Yécora, 12.4 E of Yecora on Mex. 16, *Reina G. 98-1763* (TEX); 11 km W of Restaurant Puerto de La Cruz, *Trauba 83-98* (TEX).

ACKNOWLEDGEMENTS

My long time editorial assistant, Jana Kos, reviewed the paper, for which I am grateful. Tom Van Devender provided helpful input and the picture of his professional spouse.

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Fig. 1. *Stevia reinana* (holotype).

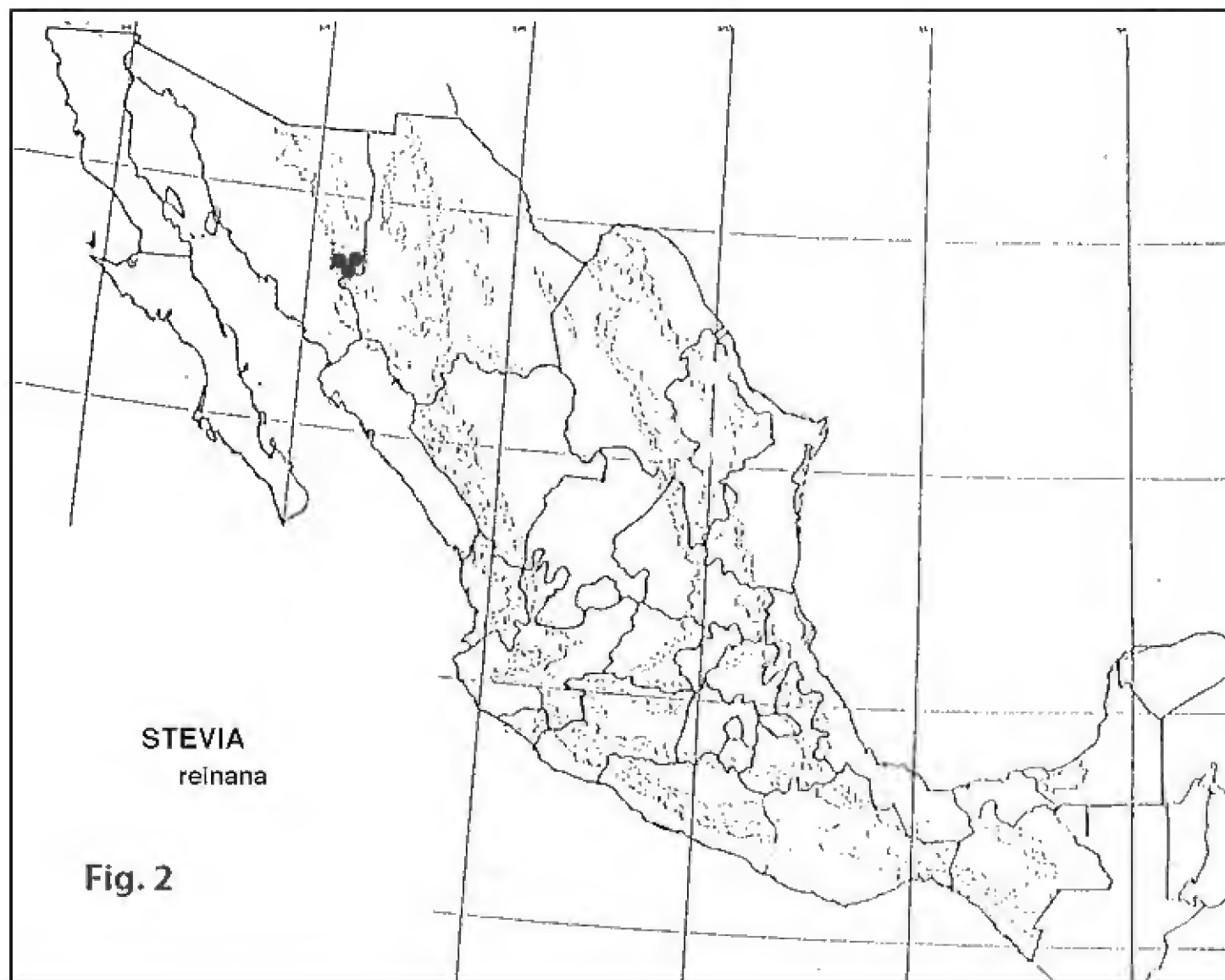


Fig. 2. Distribution of *Stevia reinana*.

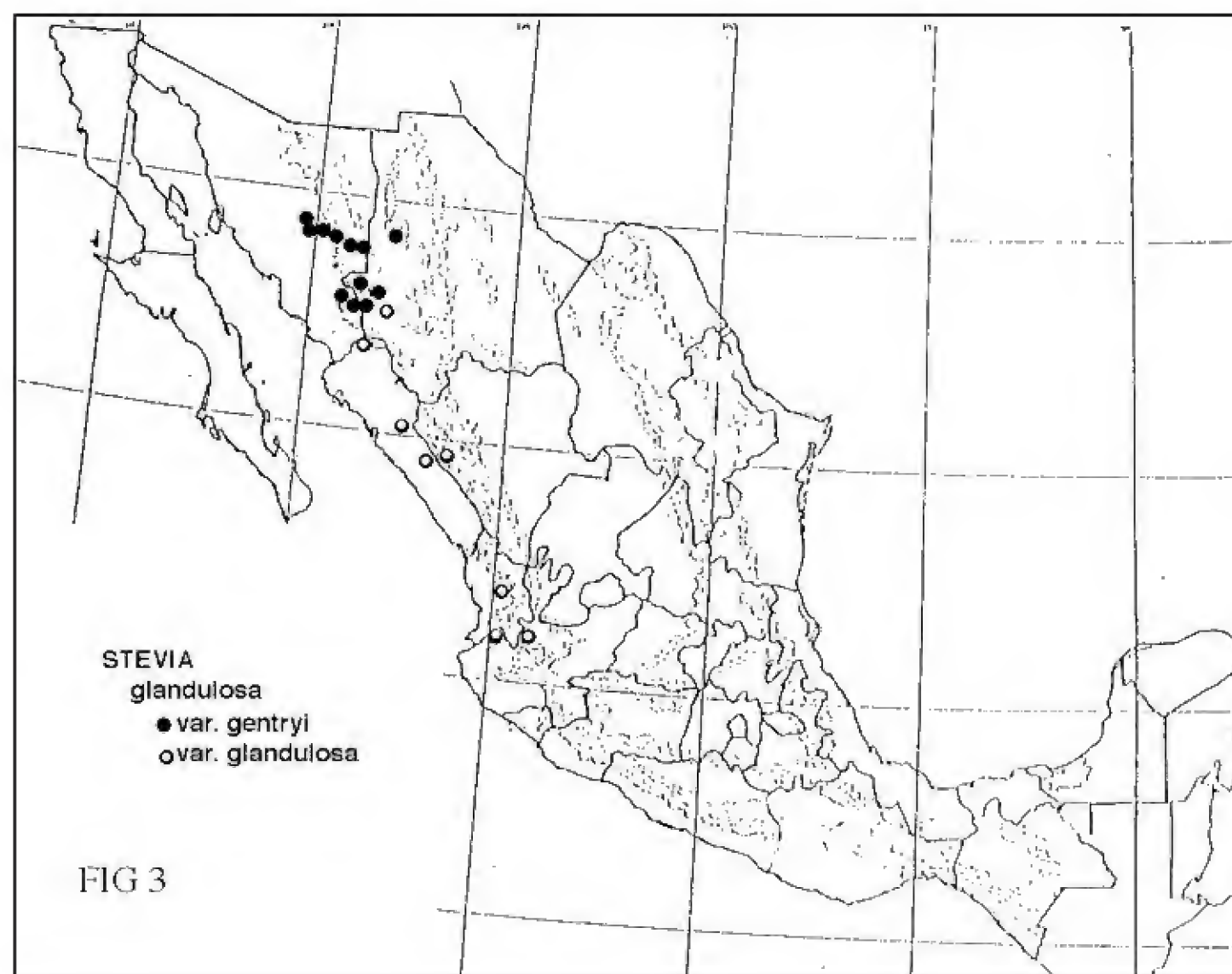


Fig. 3. Distribution of *Stevia glandulosa*.



Fig. 4. Mrs. Ana Lilia Reina-Guerrero.

Goats and deer do not use terpenoids to select or avoid browsing on *Juniperus pinchotii* Sudw. trees**Robert P. Adams**

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ABSTRACT

A comparison between heavily browsed and non-browsed *Juniperus pinchotii* trees revealed that, in contrast to the recent *J. ashei* study (Adams et al., 2013), the percentage of total volatile leaf oil yields were not significantly different ($P > 0.05$) between non-browsed trees (1.08%, DM-basis) and browsed trees (0.94%, DM basis). Only one terpene in the mg/g data, α -pinene, was significantly different ($P \leq 0.05$) between browsed and non-browsed trees. Only citronellol was significantly different ($P \leq 0.05$) in the percent total oil data. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were highly significantly different ($P \leq 0.05$) between browsed and non-browsed samples. In contrast, no significant differences ($P > 0.05$) were found for crude protein (CP), extractable condensed tannins (ECT), protein-bound tannins (PCT), total % condensed tannins (TCT), and *in vitro* dry matter digestibility (IVDMD). A comprehensive analysis of the leaf oil of *J. pinchotii* is reported. Published on-line www.phytologia.org *Phytologia* 95(3): 238-245 (August 1, 2013).

KEY WORDS: *Juniperus pinchotii*, goats, deer, browsing, terpenes, fiber, condensed tannins, digestibility, diet selection.

Recently, we reported on differences between browsed and non-browsed trees of *Juniperus ashei* Buch. (Adams et. al., 2013). In that study, we found that the yields of volatile leaf oil to be the major factor associated with browsed and non-browsed samples, with browsed trees being much lower (2.18% DM) than non-browsed trees (3.47% DM). Associated with oil yields were 12 terpenoids, all with larger mg/g in the non-browsed trees. However, the profile of terpene composition was not very effected, as only 3 terpenoids were significantly different ($P \leq 0.05$); as a percent of total oil, two of these declined in the non-browsed trees (p-cymene, 2.66, 2.18%, and semperviol, 1.66, 1.03%). Limonene increased in the non-browsed trees from 8.31 to 10.41%. It appears that selection was based mostly on the total oil yield rather than individual oil components. Of the other variables investigated: extractable condensed tannins (ECT); protein bound CT (PCT); fiber bound CT; percent total condensed tannin (TCT); percent crude protein (CP); % neutral detergent fiber (NDF); acid detergent fiber (ADF); *in vitro* dry matter digestibility (IVDMD), only PCT and IVDMD showed significant differences.

The genus *Juniperus* contains species with a large array of terpenoids and other secondary compounds (Adams, 2011). It is common to find trees that have been browsed by deer (as well as domestic goats and sheep). Schwartz et al. (1980a) observed browsing by deer and then tested confined deer in feeding trials using foliage of *J. deppeana*, *J. monosperma* and *J. scopulorum*. They found that

the consumption of juniper foliage varied inversely with the total oil yields among these three species. Schwartz et al. (1980a) also found that the levels of oxygenated terpenoids were a greater feeding deterrent than the amount of hydrocarbon terpenoids. Recently, Marko et al. (2008) reported that leaf essential oil concentrations were lowest in *J. communis* when heavily browsed by sheep and rabbits, and highest in non-browsed plants.

Juniper foliage intake by goats is limited by the presence of monoterpenes (Riddle et al., 1996; Pritz et al., 1997). Monoterpenes have a clearly defined ecological defensive role as feeding deterrents in a variety of mammals and insects (Gershenzon et al., 1992). Negative post-ingestive consequences experienced by large ungulates following consumption of high levels of monoterpenes include rumen microbial inhibition (Oh et al., 1967; Schwartz et al. 1980b), hepatic pathogenesis (Straka, 1993; Bisson et al., 2001; Pritz et al., 1997), and feeding cessation (Dziba et al., 2006). Furthermore, the presence of condensed tannins (CT) in plant leaves has been associated with protection against herbivory (Feeny, 1976) by inducing post-ingestive feedback (Provenza, 1995). The strength or direction of this feedback depends on individual plant-herbivore characteristics and, therefore, requires individual situation-testing (Stamp, 2003). The objective of the present study was to correlate plant chemical and nutritive values in *J. pinchotii* leaves with the incidence of browsing by goats (and to a much lesser extent, deer).

MATERIALS AND METHODS

Study Site - The study was conducted at the Texas AgriLife Research Station, Sonora, located on the southwestern edge of the Edwards Plateau (30° 15.747' N, 100° 34.164' W, 707 m). Annual precipitation averages approximately 600 mm; it is bimodal, with largest amounts occurring in spring and fall. Soils in the study pasture are Tarrant silty clays; soil depth overlaying a fractured limestone substrate ranges from about 10 to 450 mm. Dominant herbaceous species include *Hilaria belangeri* (Steud.) Nash and *Bouteloua curtipendula* (Michx.) Torr. Dominant woody species include *Quercus fusiformis* Mill., *Q. pungens* Liebm. var. *vaseyana*, *J. ashei* and *J. pinchotii*. Fires within the area have not occurred for at least 100 years before data collection. From 1983 to 1993, stocking rates in the study pasture were maintained at a moderate level (i.e., 11.3 ha/animal). From 1994, stocking rates in the study pasture have varied from 18 to 10.4 ha/animal. A combination of cattle, sheep, and goats were grazed on the pasture until 2003 when all cattle were removed from the study area. The area is currently grazed by goats and deer.

Plant material - Ten browsed *J. pinchotii* trees were randomly selected along a serpentine transect line of approximately 200 m. Trees showing severe browsing (e.g., all lower branches up to approximately 1 m were essentially defoliated) and having new, immature growth on the browsed branches were sampled as "browsed trees". This corresponds to the 'heavily browsed' category of Marko et al. (2008, Fig. 2, right). Ten trees with no evidence of browsing were sampled along the transect as 'non-browsed' trees, corresponding to the 'non-browsed' category of Marko et al. (2008, Fig. 2, left). Trees with other levels of browsing were excluded from sampling. *Juniperus pinchotii* has two kinds of leaves: whip- (decurent) and scale-like leaves that remain functional for 4-6 years. The whip leaves are only found on the new growth (leaders). At the study site, due to drought, very few whip-leaves were observed and the occasional branch with whip-leaves was excluded from sampling, as Adams and Hagerman (1976) found significant differences in the oils from whip- and scale-like leaves of *Juniperus*. No samples were taken from the branches with new, immature whip-leaves. Careful attention was given to sample at least 1 m above the browse line. As the browse line was up to 1 m above the ground, samples were taken at 2 to 2.5 m heights from both non-browsed and browsed trees from the south side. All trees were similar in size and height (3 to 6 m). Foliage was collected on Nov. 5, 2012. Specimens collected: *Juniperus pinchotii*, browsed trees (Adams 13613-13622) and non-browsed trees: (Adams 13623-13632); herbarium vouchers are deposited in the herbarium, Baylor University (BAYLU).

Essential oils analysis - A portion (200 g FW) of the fresh foliage was kept cool (20°C) and in the dark, then, within 24 hr, exhaustively steam-distilled for 24 h using a modified circulatory Clevenger-type apparatus (Adams, 1991). Oil samples were concentrated (diethyl ether trap-removed) with nitrogen and stored at -20°C until analyzed. Steam distilled leaves were oven dried to a constant weight (48 hr, 100°C) for the determination of oil yield as [oil wt. / (oil wt. + oven dried extracted foliage wt.)]. The extracted oils were analyzed on a HP5971 MSD mass spectrometer: 0.2 µl of a 10% solution (in diethyl ether) oil injected, split, 1:10, temperature programmed, linear, 60° - 246°C at 3°C/min. (62 mins.), carrier gas He, flow 34.96 cm/sec or 1.02 ml/min, injector 220°C, detector 240°C, scan time 1/sec, directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25-micron coating thickness, fused silica capillary column (see Adams, 2007, p. 4, for detailed operating conditions). Identifications were made by searches of our volatile oil library (Adams, 2007) using HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantification was by flame ionization detector on an HP 5890 gas chromatograph operated under the same conditions as the GCMS (above) using the HP Chemstation software.

Condensed tannin analysis - Condensed tannins in air dried (48 hr, 42°C) leaves were assayed for ECT, PCT, and FCT fractions by methods described by Terrill et al. (1992). Samples were oven-dried and standards prepared from Ashe juniper as recommended by Wolfe et al. (2008).

Crude protein, fiber and in vitro dry matter digestibility analyses - A portion of each foliage sample was air dried (48 hr, 55°C), ground in a Wiley Mill (Arthur H. Thomas Co., Philadelphia, PA) to pass a 1-mm screen, and analyzed for N (AOAC, 2006); CP was calculated as $6.25 \times N$. An additional 0.35 g of the ground sample material was placed into separate F57 digestion bags and analyzed for 48-hr true IVDMD using an Ankom Daisy II incubator (Ankom Technol. Corp., Fairport, NY). Bags were placed into an incubation jar containing 400 mL of goat rumen fluid (donors fed Tifton 85 hay) and 1,600 mL of McDougal's buffer solution (1.064 g urea L⁻¹). After anaerobic incubation at 39°C, all bags were gently rinsed under cold water and washed by hand until water was clear. Bags were subjected to the neutral detergent fiber procedure according to Van Soest et al. (1991), modified for an Ankom 2000 Fiber Analyzer (Ankom Technol. Corp., Fairport, NY) without correcting for residual ash and using α -amylase and Na sulfite. Bags were then rinsed in acetone and dried at 55°C in a forced-air oven for 48 hr and weighed.

Statistical analyses - Terpenoids (as percentage of total oil and as mg per g dry foliage weight) compared among the samples by ANOVA and SNK (Student-Newman-Keuls) analyses as described by Steele and Torrie (1960). Gower or Manhattan metric (Gower, 1971; Adams, 1975) were computed among all populations using character weighting of F-1 (F from ANOVA). Principal Coordinate Ordination (PCO) was performed to examine the patterns of association among browsed and non-browsed trees (formulation of Gower, 1966 and Veldman, 1967). Differences were considered significant at $P \leq 0.05$, unless otherwise stated.

RESULTS AND DISCUSSION

A comprehensive analysis of the leaf oil of *J. pinchotii* is shown in Table 1. In contrast to a recent *J. ashei* study (Adams et al., 2013), the percentages of total volatile leaf oil yields were not significantly different between non-browsed trees (1.08%, DM-basis) and browsed trees (0.94%, DM-basis; Table 1). This result is also in contrast to the findings of Marko et al. (2008) who found that sheep and rabbit-browsed *J. communis* trees in Hungary had lower total volatile leaf oils than non-browsed trees. Only one terpene in the mg/g data, α -pinene, was significantly different between browsed and non-browsed (Table 1). Likewise, only citronellol was significantly different in the percent total oil data (Table 1). Although no difference in total oils was observed, physiological stress factors due to prolonged drought period may have influenced grazing preference at the tree sites sampled. Also, it is possible that

the time when the trees were browsed, versus when samples were collected, may have limited the ability to accurately detect differences in total oil and terpene composition.

Neutral detergent fiber (NDF) was highly significantly different between browsed and non-browsed samples (Table 1). Acid detergent fiber (ADF) was also significantly likewise different between samples (Table 1). In contrast, no significant differences were found (Table 1) for CP, ECT, PCT, TCT, and IVDMD.

These data, on contrasting browsed and non-browsed *J. pinchotii*, present a different picture of juniper browsing than found in *J. ashei*. However, these two junipers present quite different challenges to goats (and deer). *Juniperus ashei* was shown (Adams et al., 2013) to have two kinds of leaf oil levels in the population: trees with low amounts of oil (2.15%) and trees with 62% greater oil (3.47%). In contrast, oil levels in *J. pinchotii* were not significantly different. Thus, selection by goats or deer for low-oil trees was not possible. In addition, the oil compositions were uniform in this *J. pinchotii* population, so browsers did not select for trees particularly low (or high) in some terpene.

The differences found in fiber concentration (NDF, ADF) may not indicate that goats are selecting for greater fiber. They may be selecting for some mineral or chemical with a strong taste or odor. This unknown factor may be correlated with fiber concentration. Of course, it is possible that goats were selecting from more tender foliage and that was correlated with increased yields of fiber.

To examine the pattern of browsed and non-browsed trees, Principle Coordinate Ordination (PCO) was performed on several sets of data. The PCO using all 36 characters (Fig. 1) shows a random intermixing of browsed and non-browsed trees. Utilizing only the 25 terpenoids (including oil yield) reveals a somewhat different pattern (Fig. 2), with two trees having rather distinct terpenes, but the browsed and non-browsed are still intermingled. A tendency for more of the browsed trees to register to the left of the ordination, based on 25 terpenoids (Fig. 2), is apparent.

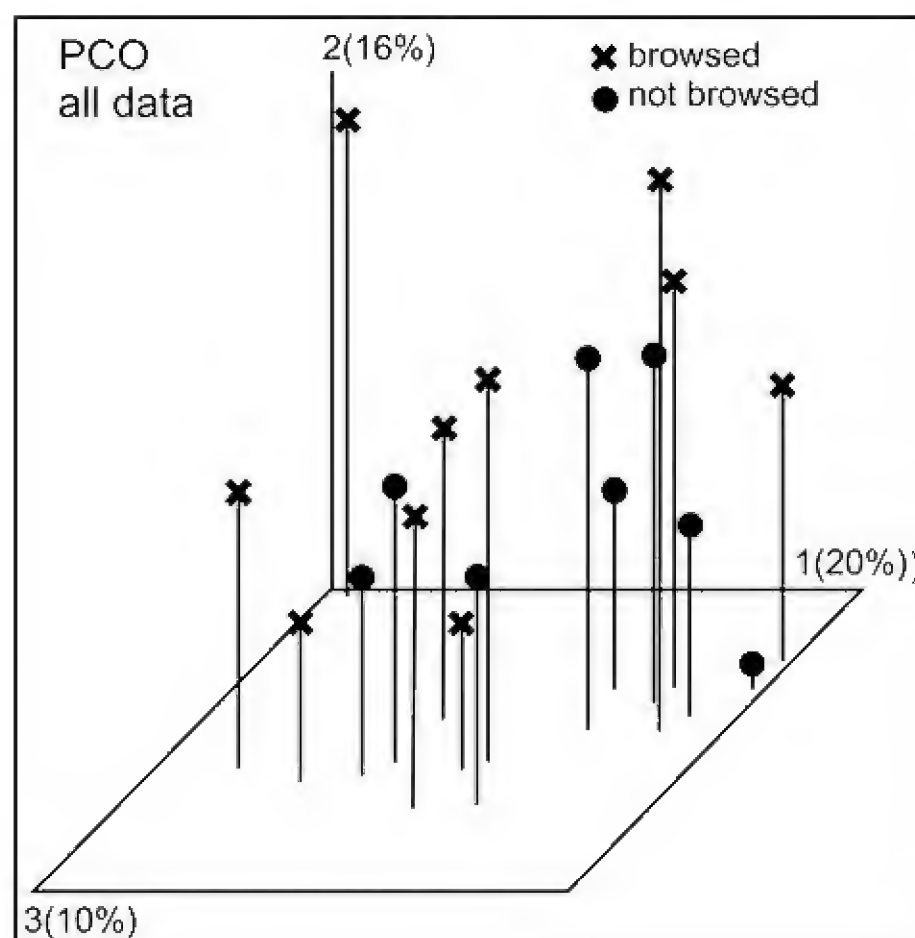


Figure 1. Principle Coordinate Ordination (PCO) based on all data.

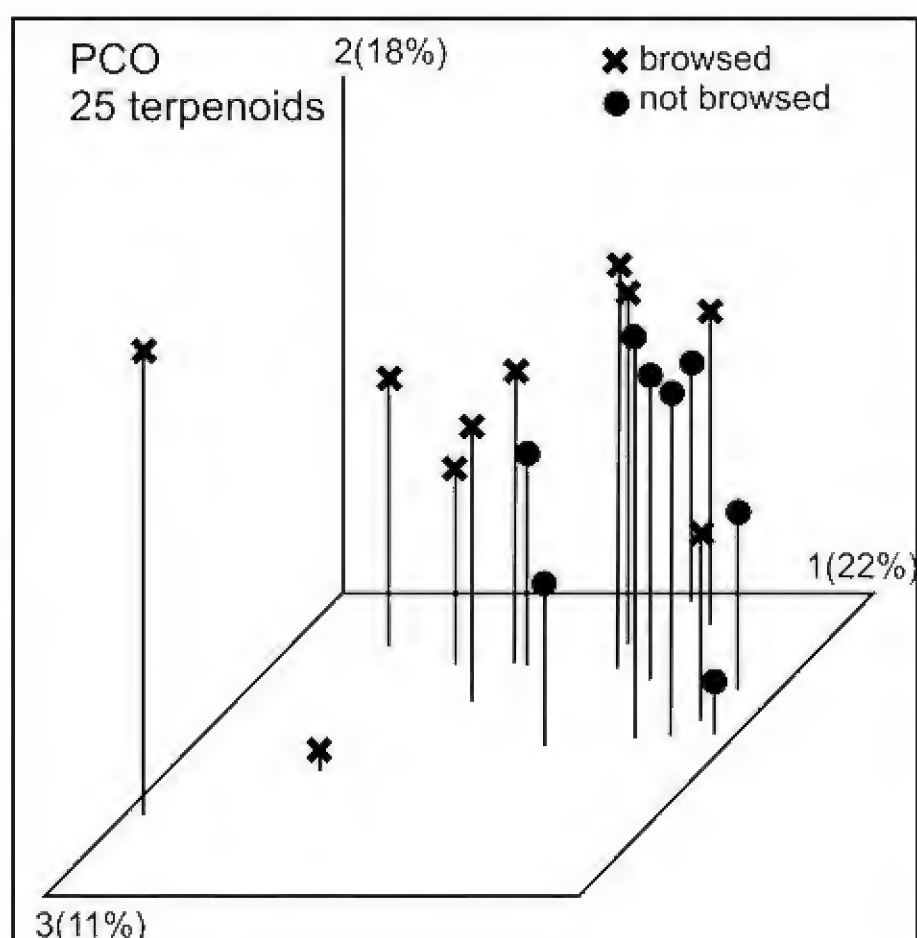


Figure 2. PCO based on only the terpenoids (25).

PCO based on only the 7 fiber, tannin, protein and digestibility characters (Fig. 3) fails to show any pattern of clustering by browsed/ non-browsed groups.

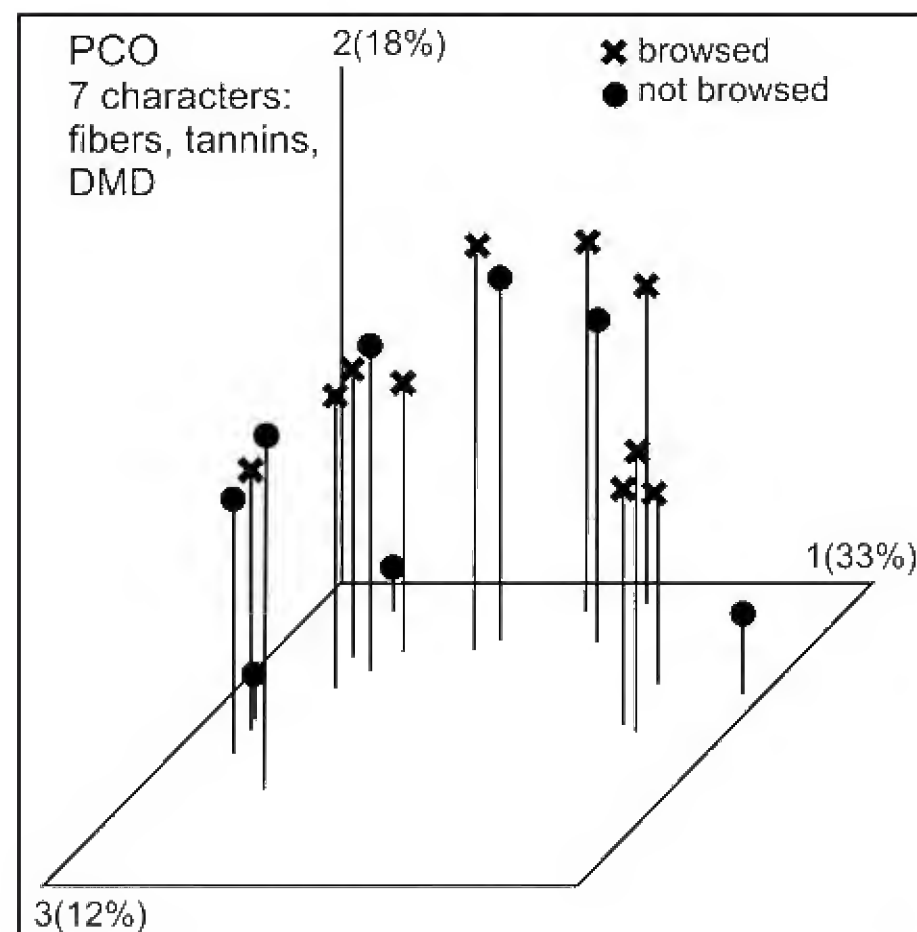


Figure 3. Principle Coordinate Ordination (PCO) based on neutral detergent fiber, acid detergent fiber, crude protein, extractable condensed tannins, protein-bound condensed tannins, total condensed tannins and in vitro dry matter disappearance.

Finally, it should be noted that goats do not browse *J. pinchotii* as readily as *J. ashei* and since goats are sociable animals that congregate under trees that may be marked by odors (urine, etc.) it may be that initial browsing is nothing more than taking a bite from the tree and this is followed by other goats until a browsing pattern is established; thence a particular the tree gets occasional heavy browsing by chance rather than design.

ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University and Texas A&M AgriLife Research.

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Table 1. Comparison of leaf oils obtained from browsed and not browsed trees of *Juniperus pinchotii*. brow% = browsed, % total oil data; nbrow% = not browsed, % total oil data; brow mg/g = browsed, mg/g DW data; nbrow mg= not browsed, mg/g DW data; F sig = F ratio and significance, $P \leq 0.05 = *$; $P \leq 0.01 = **$, ns = non significant, nt = not tested. Factors with significant differences are in boldface.

	Factor tested	browsed %	nbrowsed %	F sig	brow mg/g	nbrow mg/g	F sig
	oil yields (% ODW; mg/g ODW)	0.941	1.083	3.71 ns	9.41	10.08	3.68 ns
	neutral detergent fiber(NDF)	40.05	36.86	8.61 **			
	acid detergent fiber(ADF)	28.17	26.37	5.66 *			
	crude protein	7.44	7.31	0.35 ns			
	extractable condensed tannins	6.84	6.30	0.79 ns			
	protein bound tannins	3.40	3.06	1.06 ns			
	total % condensed tannins	10.24	9.35	1.11 ns			
	<i>in vitro</i> dry matter digestibility	64.92	64.33	0.25 ns			
KI	Compound	brow%	nbrow%	F sig	brow mg/g	nbrow mg/g	F sig
921	tricyclene	0.30	0.33	nt	0.03	0.04	nt
924	α -thujene	0.84	0.96	0.23 ns	0.08	0.10	ns
932	α-pinene	1.22	1.31	0.01 ns	0.11	0.14	4.76 *
946	camphene	0.45	0.46	nt	0.05	0.05	nt
969	sabinene	24.18	25.42	0.36 ns	2.27	2.75	2.50 ns
974	β -pinene	0.12	0.11	nt	0.01	0.01	nt
988	myrcene	3.07	2.99	0.15 ns	0.29	0.32	2.45 ns
1002	α -phellandrene	0.10	0.10	nt	0.01	0.01	nt
1008	δ -3-carene	0.11	0.18	nt	0.01	0.02	nt
1014	α -terpinene	1.92	1.99	0.01 ns	0.18	0.22	2.52 ns
1020	p-cymene	0.16	0.21	nt	0.02	0.02	nt
1024	limonene	3.21	3.10	0.17 ns	0.30	0.33	1.01 ns
1025	β -phellandrene	2.20	2.11	0.12 ns	0.21	0.23	0.71 ns
1054	γ -terpinene	3.21	3.24	0.67 ns	0.30	0.35	2.36 ns
1065	cis-sabinene hydrate	1.45	1.49	0.17 ns	0.14	0.16	3.76 ns
1086	terpinolene	1.44	1.38	0.78 ns	0.14	0.15	2.59 ns
1098	trans-sabinene hydrate	1.03	1.15	0.97 ns	0.09	0.12	3.79 ns
1099	linalool	0.61	0.67	0.01 ns	0.06	0.07	1.37 ns
1118	cis-p-menth-2-en-1-ol	0.56	0.49	0.39 ns	0.06	0.05	3.05 ns
1141	camphor	24.49	25.36	0.07 ns	2.30	2.74	1.53 ns
1145	camphene hydrate	0.69	0.73	0.21 ns	0.07	0.07	1.36 ns
1148	citronellal	0.92	0.77	0.54 ns	0.09	0.08	0.94 ns
1165	borneol	0.66	0.74	0.14 ns	0.06	0.08	0.96 ns
1174	terpinen-4-ol	7.38	7.66	0.29 ns	0.70	0.82	4.14 ns
1186	α -terpineol	0.36	0.41	nt	0.04	0.04	nt
1195	cis-piperitol	0.16	0.11	nt	0.02	0.01	nt
1207	trans-piperitol	0.21	0.22	nt	0.02	0.02	nt
1219	coahuilensol, me-ether	0.10	0.10	nt	0.01	0.01	nt
1223	citronellol	4.78	3.14	5.22 *	0.45	0.34	3.20 ns
1274	pregeijerene B	0.14	0.13	nt	0.01	0.01	nt
1284	bornyl acetate	1.68	1.69	0.01 ns	0.16	0.18	0.26 ns
1289	thymol	0.11	0.10	nt	0.01	0.01	nt
1389	β -elemene	t	t	nt	<.01	<.01	nt
1451	trans-muurolo-3,5-diene	0.10	0.17	nt	0.01	0.02	nt
1475	trans-cadina-1(6),4-diene	0.08	0.11	nt	0.01	0.01	nt
1493	trans-muurolo-4,5-diene	0.23	0.40	nt	0.02	0.04	nt
1500	α -muurolene	t	t	nt	<.01	<.01	nt
1514	cubebol	0.27	0.42	nt	0.03	0.04	nt
1522	δ -cadinene	0.11	0.21	nt	0.01	0.02	nt
1528	zonarene	t	t	nt	<.01	<.01	nt
1548	elemol	5.81	3.97	ns	0.55	0.43	1.17 ns
1627	1-epi-cubenol	0.26	0.37	nt	0.03	0.04	nt
1630	γ -eudesmol	0.53	0.37	3.02 ns	0.05	0.04	2.36 ns

KI	Compound	brow%	nbrow%	F sig	brow mg/g	nbrow mg/g	F sig
1649	β -eudesmol	0.80	0.60	2.01 ns	0.08	0.06	0.89 ns
1652	α -eudesmol + α -cadinol	0.80	0.59	2.24 ns	0.08	0.06	0.86 ns
1670	bulnesol	0.32	0.23	nt	0.03	0.02	nt
1792	8- α -acetoxylemol	t	t	nt	<.01	<.01	nt
1987	manoyl oxide	0.16	0.23	nt	0.01	0.02	nt
2055	abietatriene	t	t	nt	<.01	<.01	nt
2087	abietadiene	t	t	nt	<.01	<.01	nt
2298	4-epi-abietal	0.13	0.20	nt	0.01	0.02	nt

Two new species of *Brickellia* (Asteraceae: Eupatorieae) from Mexico

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ABSTRACT

Two new species are described from Mexico, *Brickellia atarjea* B.L. Turner, **sp. nov.**, (Guanajuato, Queretaro and Hidalgo), and *Brickellia serboana* B.L. Turner, **sp. nov.**, of Oaxaca. The former is related to the widespread *B. secundiflora*, the latter to *B. orizabensis*. Photographs of the types are provided, along with distribution maps of the taxa concerned. Published on-line www.phytologia.org *Phytologia* 95(3): 246-251 (August 1, 2013).

KEY WORDS: Asteraceae, Eupatorieae, *Brickellia*, Mexico, Guanajuato, Hidalgo, Queretaro, Oaxaca

Routine identification of Mexican Asteraceae has revealed the following novelties:

BRICKELLIA ATARJEA B.L. Turner, **sp. nov.** Fig. 1

Resembling *Brickellia secundiflora* (Lag.) A. Gray but the heads fewer, larger (ca. 2 cm high and as wide), more numerous florets (40-60 vs 8-25) on longer peduncles, the outer involucre bracts broadly ovate, these set off from the more linear-lanceolate innermost series.

Stiffly erect suffruticose herbs, 0.5-1.0 m high. **Mid-stems** crinkly-pubescent with short hairs 0.2-0.6 mm high, or densely glandular-pubescent throughout. **Leaves** broadly ovate to cordate, 5-7 cm long, 3.5-5.0 cm wide; petioles mostly 5-12 mm long; blades markedly reticulate-venose beneath, coarsely pubescent throughout, mainly along the veins, the margins crenulate. **Heads** axillary, 1-3 to a node, the ultimate peduncles 1-5 cm long, crinkly-pubescent or glandular-pubescent. **Involucres** 6-7 seriate, markedly imbricate, ca 15 mm high, the outer series broadly ovate, grading into the more lanceolate inner series. **Receptacles** plane to convex, 2.5-4.0 mm across, pubescent with white hairs 0.5-1.5 mm long. **Florets** 40-60 (estimated); corollas slender, 8-9 mm long, reportedly “amarillas,” “blancas,” or “moradas,” the lobes ca 0.5 mm long. **Styles** with bases enlarged, densely pubescent; branches slender, ca 5 mm long. **Achenes** 9-ribbed, 5-6 mm long, pubescent throughout with appressed hairs; pappus of 20-25 short-pectinate awns or bristles 7-9 mm long.

TYPE: MEXICO. GUANAJUATO: Mpio. de Atarjea, Cerro Pichardo, “bosque de pino, ladera de cerro,” 2100 m, 2 Oct 1990, *E. Ventura* y *E. Lopez* 8842 (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: MEXICO. HIDALGO: Mpio. Ajacuba, Cerro Los Aguilares, NW del poblado Santiago Tezontlale, Sierra del “Mexe,” 2650 m, 4 Nov 1989, *Diaz* 724 (TEX). **QUERETARO: Mpio. San Joaquin**, “Ruinas las Ranas,” 2 km al N de San Joaquin, 2300 m, 14 Nov 1993, *Fernandez N.* 4867 (TEX).

Brickellia atarjea will key to or near *B. secundiflora* in my treatment of *Brickellia* for Mexico (Turner 1997). The several sheets described herein are readily distinguished from the latter by their larger heads on mostly longer ultimate peduncles. It is possible that the Fernandez collection from Queretaro represents an undescribed taxon since the pubescence is predominantly glandular-pubescent (spreading crinkly hairs mostly absent), and the specimen has heads that are less markedly biseriate (the outer series grading more perceptively into the inner series); conversely, the specimen might also prove to be a hybrid derivative of *B. secundiflora*, populations of which occur in the area. Regardless, all of the cited sheets

differ significantly from the large number of other sheets of *B. secundiflora* (including its 4 varieties) on file at LL-TEX (as mapped by Turner 1989). Distributions of the two sympatric taxa concerned are shown in Fig. 3 and Fig. 4.

The name derives from the Municipio de Atarjea, Guanajuato, whence the type.

BRICKELLIA SERBOANA B.L. Turner, *sp. nov.* **Fig. 2**

Resembling *Brickellia orizabensis* Klatt but the leaves ovate-lanceolate, 3-5 times as long as wide (vs broadly ovate, 1-2 times as long as wide; petioles 3-6 mm long (vs 10-50 mm), florets mostly 25-30 per head (vs 15-20); achenes viscid, pubescent with minute glandular trichomes (vs not so).

TYPE: MEXICO. OAXACA: Mpio. Santo Domingo Tonola, Paraje “Amates Amarillos” Guamil, sobre cerro, ca 1634 m, 17 39 57.7 N, 97 56 36.6 W, 15 Oct 2008, *Armando Torres Hernandez* 499 [con L. Hernandez]. (Holotype: TEX).

Suffruticose herbs, or shrublets to 1 m high. **Mid-stems** ca 4 mm thick, purplish, glabrate. **Leaves** mostly opposite (rarely not), 3-7 cm long, 1-3 cm wide; petioles 3-6 cm long, more or less winged; blades ovate-lanceolate, glabrous, weakly 3-nerved near the base. **Capitulescence** a terminal, numerous-headed, cymose-panicle ca 30 cm high, 20 cm across. **Heads** (in fruit) ca 15 mm high, 20 mm across, the peduncles mostly 5-10 mm long, minutely puberulent with up curved hairs. **Involucral bracts** glabrous, 2-3 seriate, gradate, the inner series oblanceolate, 5-7 mm long, apices acute to obtuse. **Receptacle** plane, epaleate, glabrous, ca 3 mm across. **Florets** 25-30 per head; corollas white, 5-6 mm long, glabrous, 5-lobed. **Achenes** ca 5 mm long, 8-9 ribbed, both viscid and pubescent with minute glandular trichomes, mostly along the ribs; pappus of ca 45 tawny, ciliate, bristles 5-8 mm long.

In my treatment of *Brickellia* for the Comps of Mexico (Turner 1997), this novelty will not key to a given taxon, but it will “feather-out” somewhere in the vicinity of *B. orizabensis*. It is similar to the latter in leaf arrangement, habit and capitulescence, but differs markedly in having ovate-lanceolate, glabrous leaves with very short winged petioles, and remarkably viscid achenes, these possessing minute glandular-trichomes, as called to the fore in the above diagnosis. Distribution of the two taxa is shown in Fig. 5.

The name is an anagram derived from Sociedad para el Estudio de los Recursos Bioticos de Oaxaca (SERBO), who provided funds for the assemblage concerned.

ACKNOWLEDGEMENTS

I am grateful to my research companion, Jana Kos, for editing the paper and providing helpful suggestions. Dot-maps are based upon specimens on file at LL-TEX.

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Fig. 1. Holotype of *Brickellia atarjea*.



Fig. 2. Holotype of *Brickellia serboana*.

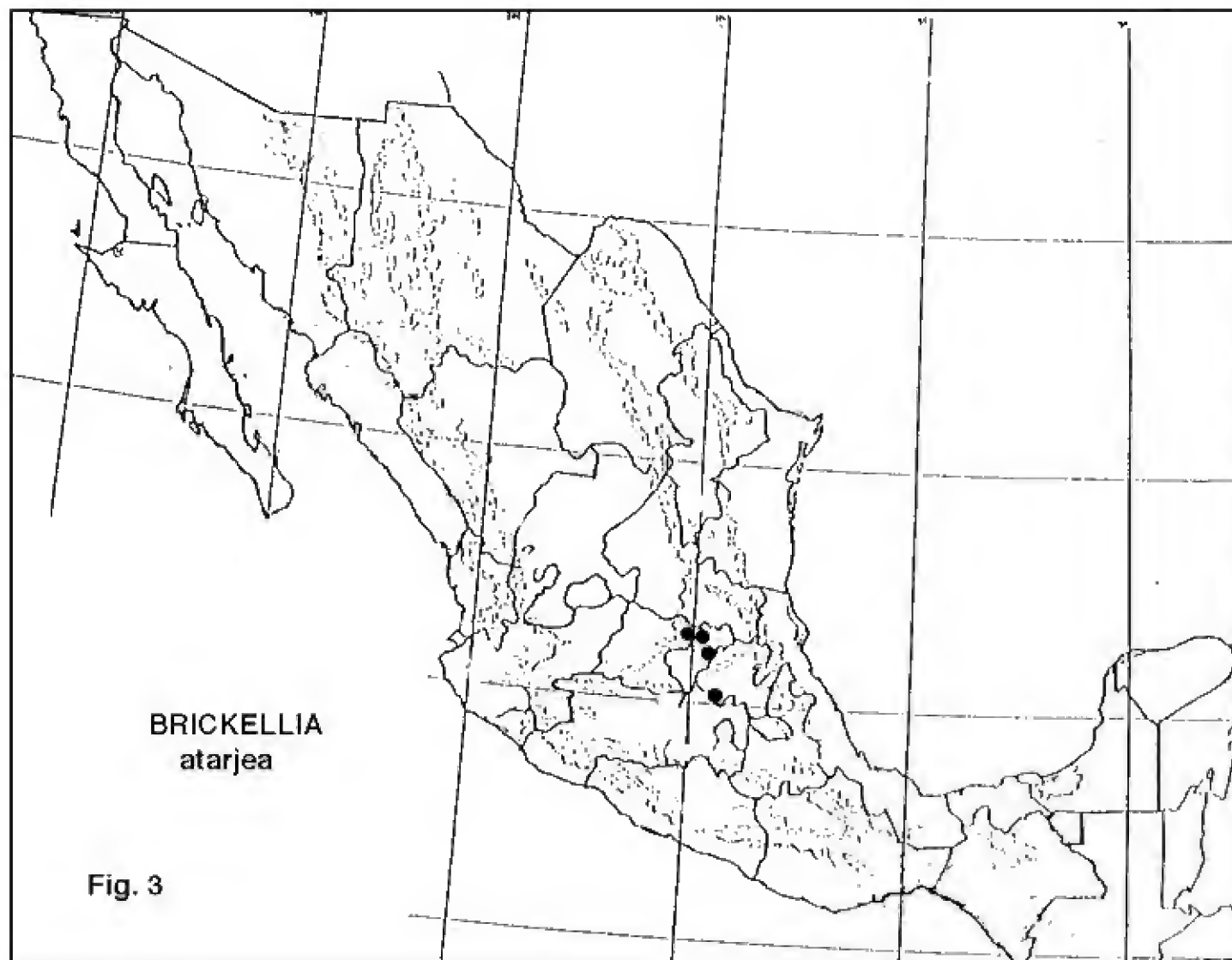


Fig. 3. Distribution of *Brickellia atarjea*.

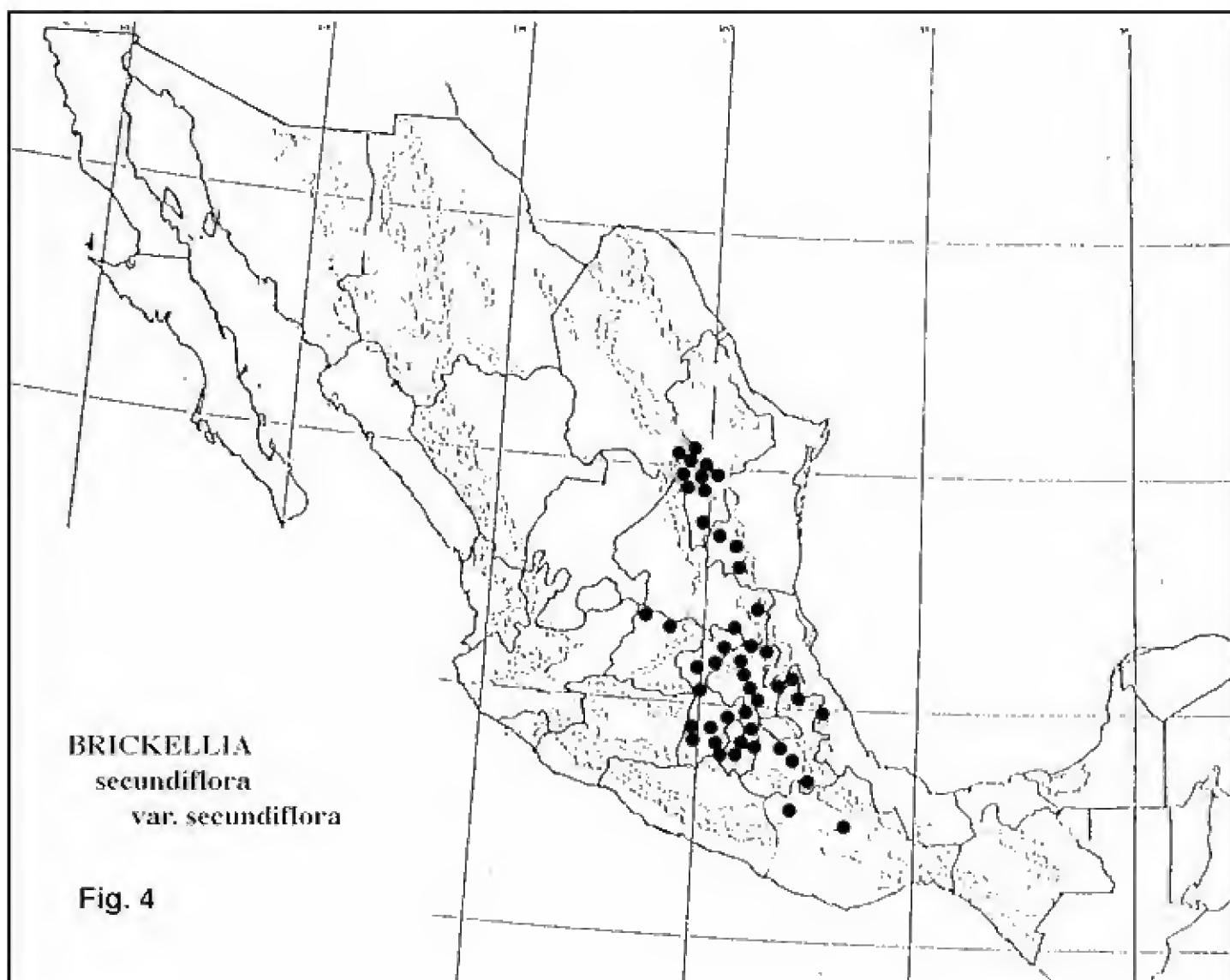


Fig. 4. Distribution of *Brickellia secundiflora* var. *secundiflora*.

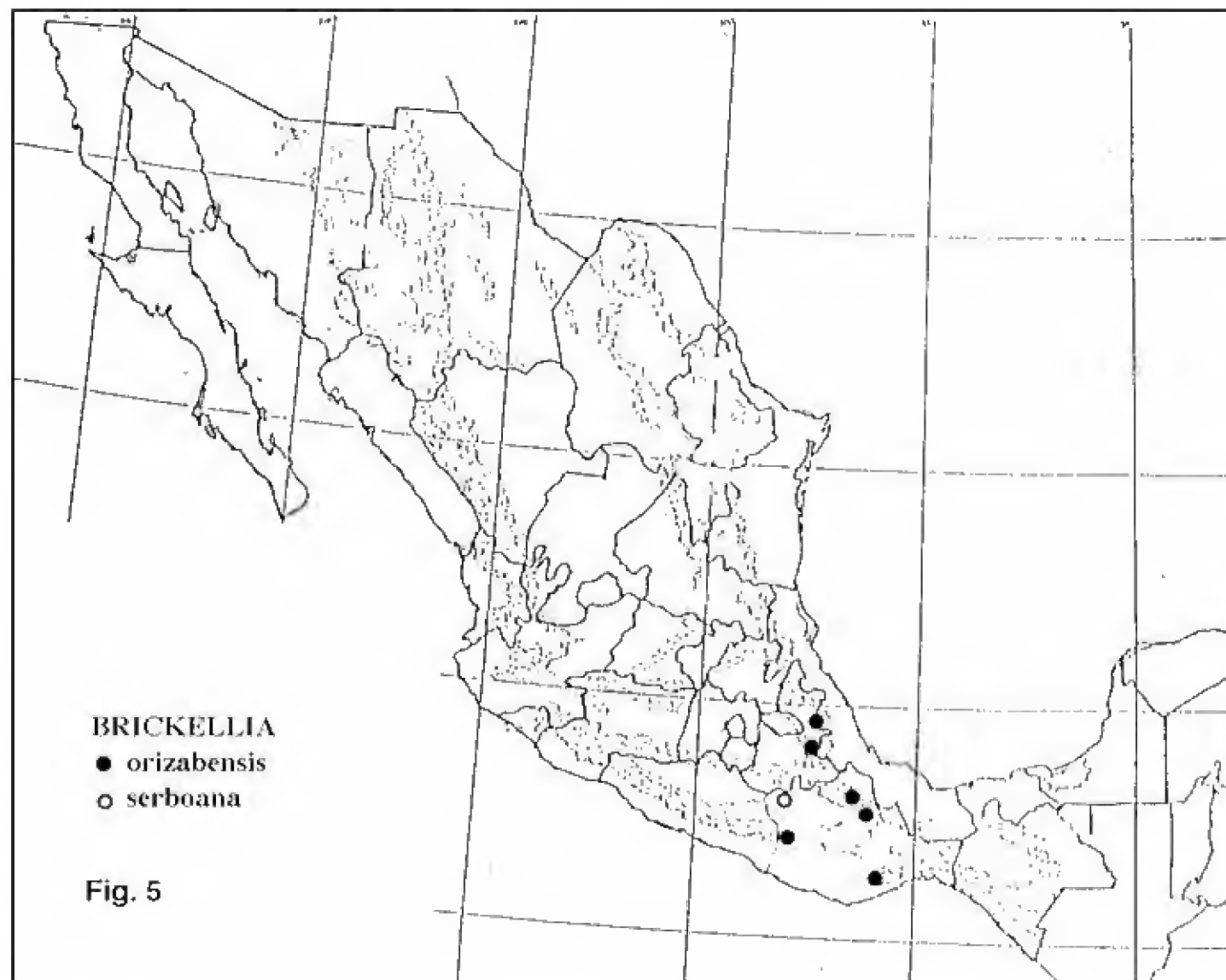


Fig. 5. Distribution of *Brickellia serboana* and *B. orizabensis*.

Lectotypification of *Oryctanthus occidentalis* (L.) Eichler (Loranthaceae)

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ABSTRACT

The lectotype of *Oryctanthus occidentalis* (L.) Eichler (Loranthaceae) is designated, correcting an earlier error. www.phytologia.org *Phytologia* 95(4): 248-249 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Lectotypification, *Loranthus occidentalis*, *Oryctanthus occidentalis*.

The mistletoe species, *Oryctanthus occidentalis* (L.) Eichler, originally described as *Loranthus occidentalis* L. from Jamaica, has subsequently been recognized as mostly a continental taxon. Kuijt (1976) treated a specimen housed in the Sloane Herbarium (BM) as the holotype of this species; he, however, was uncertain whether Linnaeus saw the specimen (“whether or not Linnaeus saw this specimen”). Kuijt’s assessment, as also indicated by Jarvis (2007), is incorrect, for Linnaeus never examined the Sloane Herbarium specimens. [“It is well known that Linnaeus did not examine these specimens but rather used the Catalogue, and the illustrations published in the History, to authenticate many of the 1753 binomials which included a reference to a Jamaican plant. Linnaeus reproduced Sloane’s polynomials verbatim, only changing the Latin very slightly here and there.” (vide The Sloane Herbarium).] Additionally, the place of original publication of *L. occidentalis* as cited in Kuijt [1976: 520 (as “Amoen. Acad. 5: 396. 1760”)] is also incorrect: it was first published in Linnaeus’ *Systema Naturae*, ed. 10, 2: 988 (1759), where it is listed as specimen No. 2 under *Loranthus* (bottom 2 lines). Linnaeus did acknowledge having seen the figure in Sloane’s “Voyage”, erroneously citing it as “T. 100 f. 2”, which should have been “T. 200 f. 2”.

The relevant sheet in Sloane’s herbarium bears two different specimens, *Oryctanthus occidentalis* being in the center of the sheet; the other specimen is probably *Phoradendron quadrangulare* (Kunth) Griseb. (Viscaceae), a common parasite throughout most of tropical America (Kuijt 2003). Sloane’s illustration is unmistakably based on the above-mentioned *Oryctanthus* specimen.

Our conclusion, therefore, is that the above mentioned Sloane figure is to be designated the lectotype of *Oryctanthus occidentalis*. The actual specimen upon which the illustration is based cannot be regarded as the type.

Taxonomic Summary:

Oryctanthus occidentalis (L.) Eichler in Martius, Fl. Bras. 5(2): 87, t. 89. 1868.

Loranthus occidentalis L., Syst. Nat., ed. 2: 988. 1759.

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ACKNOWLEDGEMENT

We thank Kanchi N. Gandhi for pointing out the correct place of publication.

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Two Novel *Stevias* (Asteraceae: Eupatorieae) from North-Western Mexico

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ABSTRACT

Two novelties of *Stevia* are described from Mexico: *Stevia concordiana* B.L. Turner, **sp. nov.**, from Sinaloa, Mpio. Concordia, and *Stevia sahuaribana* B.L. Turner, **sp. nov.**, from the vicinity of Sahuariba, Sonora, reportedly near the waterfall Sahuariba. The latter is presumably related to the more widespread, more southern species, *S. rosei*, the former to *S. hypomalaca*, a species of central Mexico. www.phytologia.org *Phytologia* 95(4): 250-254 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Asteraceae, Eupatorieae, *Stevia*, *S. hypomalaca*, *S. rosei*, Mexico, Sinaloa, Sonora

STEVIA CONCORDIANA B.L. Turner, **sp. nov.** Fig. 1

Resembling *S. hypomalaca* B.L. Rob., but having somewhat less pubescent stems and foliage, the leaves ovate-lanceolate, 10-15 mm wide (vs oblanceolate, 4-10 mm wide).

Stiffly erect perennial herbs 40-60 cm tall. **Stems** densely pubescent with crinkly hairs, the vestiture 0.5-1.0 mm high. **Leaves** alternate, ovate-lanceolate, 3-5 cm long, 1.0-1.5 cm wide, lower surfaces densely pubescent and markedly venose; petioles 1-3 mm long, the blades weakly crenulate. **Capitulescences** cymose-paniculate, arranged both terminal and lateral, each arrangement ca 3 cm high, 2 cm wide, the ultimate peduncles 1-10 mm long. **Involucral bracts** 4-5 mm long, their apices mostly acute, pubescent like the stems. **Florets** 4 / head, 3 bearing awns, the other a crown of short scales 0.1-0.3 mm high. **Corollas** white, 4-5 mm long, sparsely pubescent; tube ca 1 mm long, grading into the throat; lobes ca 1.5 mm long. **Achenes** linear, black, glabrous, ca 3 mm long; pappus a crown of short scales ca 0.2 mm high, or topped by 3-4 bristles ca 4 mm long.

TYPE: MEXICO. SINALOA: Mpio. Concordia, “El Palmito a 8 km al oeste, bosque de *Pinus* con lase species *Engelmanii*, *Herrerai* y *Quercus*,” ca 2350 m, 17 Nov 1984, J. A. Beltran Magallanes 160 (Holotype TEX).

In my treatment of *Stevia* for Mexico (Turner 1997), largely because of its alternate, densely pubescent, markedly venose leaves, this novelty will key to or near *S. hypomalaca* B.L. Rob., a more southern taxon (Fig. 3).

STEVIA SAHUARIBANA B.L. Turner, **sp. nov.** Fig. 2

Superficially resembling *Stevia rosei* B.L. Rob., but having densely pubescent stems and leaves (vs glabrous) and much smaller involucral bracts (ca 4 mm long, vs 7-8 mm).

Perennial herbs to 50 cm high. **Stems** densely pubescent with crinkly trichomes, the vestiture ca 0.5 mm high. **Leaves** mostly opposite, sessile or nearly so; blades lanceolate, 2.5-3.0 cm long, 0.5-1.0 cm wide, densely pubescent below and above, the lower surfaces more so, markedly venose beneath, the margins nearly entire to weakly dentate. **Capitulescence** a terminal cymose panicle ca 5 cm high, and as wide, the ultimate peduncles 0-2 mm long. **Involucres** pubescent like the stems. **Florets** 4 per head, two having achenes with well-developed bristles; two with a crown of scales ca 0.5 mm high. **Corollas** white,

pubescent, ca 3 mm long, the lobes 2-3 mm long. **Achenes** black, ca 3 mm long, glabrous, the pappus as described above.

TYPE: MEXICO. SONORA: Mpio. Sahuariba, vicinity of Sahuariba waterfall, “2 km north of Sahuaribo (sic) on road to Curohui.” 27 21.2 N, 108 40 W, 1450 m, “pine-oak woodland.” 20 Aug 1992, *P.S. Martin et al. s.n.* (Holotype: ARIZ).

In my treatment of **Stevia** of Mexico (Turner 1997), this novelty will key to or near **S. rosei**, largely because of its small leaves. As noted in the above diagnosis, it is readily distinguished from the latter, more southern, species (Fig. 4), by numerous characters.

ACKNOWLEDGEMENTS

I am grateful to ARIZ for the prompt loan of 37 herbarium sheets, and to my editorial assistant, Jana Kos, for helpful suggestions. Distribution maps are based upon specimens cited by Grashoff (1972) and specimens on file at ARIZ, TEX.

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Fig. 1. *Stevia concordiana* (Holotype: TEX).



Fig. 2. *Stevia sahuaribana* (Holotype: ARIZ).



Fig. 3. Distribution of *Stevia concordiana* and *S. hypomalacca*.



Fig. 4. Distribution of *Stevia rosei* and *S. sahuaribana*.

***Gaillardia candelaria* var. *mikemoorei* (Asteraceae: Helenieae),
A novel gypsophile from Coahuila, Mexico**

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ABSTRACT

A novel gypsophile, *Gaillardia candelaria* var. **mikemoorei** B.L. Turner, **var. nov.** is described from Coahuila, Mexico. According to DNA data it is most closely related to the recently described *G. candelaria* B.L. Turner. Morphological differences between the two taxa are discussed and photographs of the types of both taxa are provided, along with a map showing the distribution of gypsophilic *Gaillardias* in the area concerned. . Published on-line www.phytologia.org *Phytologia* 95(3): 252-257 (August 1, 2013).

KEY WORDS: Asteraceae, Helenieae, *Gaillardia*, *G. candelaria*, *G. c.* var. *mikemoorei*, gypsophiles, Mexico, Coahuila

Preparation of a treatment of the Tribe Helenieae of Mexico (Turner 2013) occasioned the present contribution; unfortunately the plants concerned were forwarded to me for identification after the appearance of my tribal treatment. I reckoned these to belong to a novel taxon, closely related to *G. candelaria*, and describe it accordingly.

GAILLARDIA CANDELARIA var. **MIKEMOOREI** B.L. Turner, **var. nov.** **Fig. 1**

Resembling *Gaillardia candelaria* B.L. Turner, but the leaves predominantly basal (vs cauline); peduncles 6-10 cm long (vs 10-20 cm); outer involucre bracts linear-lanceolate, 9-10 mm long (vs lanceolate, 6-8 mm long); receptacles beset with sclerotic, persistent, conical, prickles ca 0.5 mm high (vs elongate-conical, 1.0-1.5 mm high); pappus scales 8 (vs ca 12) and lobes of the ray ligules 4-6 mm long (vs ca 2 mm).

Perennial, mostly scapose, herbs to 20 cm high, arising from well-developed, branched rhizomes. **Leaves** linear, mostly 4-8 cm long, 1-3 mm wide, possessing a single prominent mid-rib, sparsely pubescent above and below, not atomiferous glandular or punctate; base of leaves enlarged and clasping. **Peduncles**, 6-10 cm long, moderately pubescent with spreading white hairs ca 0.5 mm long. **Heads** single, 10-13 mm wide (the rays excluded), ca 1 cm high. **Involucre bracts (outer)**, 10-11, linear-lanceolate, 9-10 mm long, ca 1.5 mm wide, longer than the inner bracts, reflexed with age, white-pubescent, like the peduncles. **Receptacles** convex, 4-5 mm across, beset with 30-40, mostly markedly indurate, cone-shaped, enations ca 0.5 mm high. **Ray florets** ca 11, neuter, sterile; ligules yellow, 1.2-1.5 cm long, mostly 3-lobed, the lobes 3-nerved, 4-6 mm long, 2-3 mm wide. **Disc florets** 30-40; corollas 3.5-4.0 mm long; tubes ca 0.5 mm long; throats ca 4 mm long, 5-lobed, the apices pubescent with purplish trichomes. Stamens 5; anthers purplish, the appendages lanceolate, ca 1 mm long, glandless. **Style branches**, linear, purple, ca 2 mm long. **Achenes** (immature) ca 2 mm long, densely white-pubescent, mainly near the base; pappus of 8, linear-lanceolate, scales 5-6 mm long, their apices awned for 2-3 mm.

TYPE: MEXICO. COAHUILA: Mpio, Francisco 1 Madero, “West side of Valle de Buenavista Francisco 1. Madero” 1835 m. Gypsum hillside, 20 Sep 2012, 26° 34' 18" N, 103° 02' 17.6" W, *Hinton et al.* 29346 (Holotype: MEXU; isotypes: GBH, TEX).

According to the collector of the Type (G.S. Hinton, pers. comm.), var. **mikemoorei** was “the dominant species on the gyp” at the locality concerned.

ADDITIONAL SPECIMEN EXAMINED: **COAHUILA**: “On gypsum slopes on the W side of the Valle de Buenavista (E slopes of Sierra de los Remedios/Acatita.” 5831 ft, 26 36 43.1 N, 103 02 23.9 W, 22 Aug 2012, *Moore et al.* 2000 (TEX).

The collector of the above specimen (pers. comm.) noted that “One of the outstanding things about this species is that it branches underground, which I have never seen before in *Gaillardia*.” Moore also studied the DNA of both infraspecific taxa concluding “According to nuclear internal transcribed spacer (ITS) sequence data, *G. mikemoorei* [my delineation] forms a clade with *G. candelaria*, with little sequence variation among individuals of the two taxa.” Never the less, I had intended to describe the taxon as a new species, but its collector took a humbler attitude, my bowing to his wishes.

In the revisionary treatment of **Gaillardia** by Turner and Watson (2007), this novelty will key to or near **G. suavis**, differing from the latter in having simple, linear-lanceolate, leaves (vs pinnatifid), shorter peduncles, and receptacles with persistent, rigid, conical bristles (vs. mostly deciduous or absent). Actually, I misidentified two of the sheets cited above, taking these to be atypical, linear-leafed, forms of **G. pinnatifida**, one of these to have served as the type of a projected novelty, this illustrated (TEX), but not published; reexamination of the sheets concerned shows these to be the recently described **G. candelaria**.

According to Moore’s DNA data (pers. comm), **G. candelaria**, **G. powellii** and **G. henricksonii** form a well-supported gypsum endemic clade, this also apparent from morphological data.

The novelty is named for Prof. Mike [Michael] J. Moore, currently at Oberlin College, Ohio, and Academic son of Bob Jansen at the Univ. of Texas, Austin. Prof. Moore is an exceptional student of gypsophily among plants (cf. Moore and Jansen, 2007).

Initially, having examined the type, I proposed a tentative name honoring its collector, George Hinton. But George demurred, pointing out that Mr. Moore had called the type locality to his attention, and that he went to the gyp site largely at the latter’s urging. Such is honor among gentlemen, or should be.

It should be noted that at the time of my description of **G. candelaria**, I did not possess material of **G. mikemoorei**, nor was I able to provide an adequate description of the material on hand, the type of the former disappearing from my fold by the wile of yet another worker. Because of this, I have, below, modified the description of **G. candelaria**.

GAILLARDIA CANDELARIA B.L. Turner, var. **CANDELARIA** Phytologia Memoirs 13: 55. 2007.

Perennial herbs, 20-30 cm high. **Stems** suffruticose 10-15 cm long, their apices each producing a single head on softly tomentose peduncles, the latter 10-15 cm long. **Leaves** alternate, linear-lanceolate, sparsely puberulent to nearly glabrate, mostly 4-9 cm long, 2-3 mm wide, gradually reduced upwards and much-overlapping, their lower surfaces with a single pronounced midrib, their margins entire and somewhat enrolled. **Heads** 3.5-4.2 cm across the extended rays (as determined from label data). **Receptacles** hemispheric, variously endowed with semi-persistent, slender, conical enations mostly 0.1-1.5 mm high. **Involucres** 2-3 seriate, composed of subequal lanceolate bracts 6-8 mm long, ca. 2 mm wide, the outer series pubescent like the peduncles. **Ray florets**, ca 11, neuter; ligules yellow. 10-15 mm long, mostly 3-lobed at their apices, the sinuses 2-3 mm deep. **Disc florets** 30-50 (estimated); corollas yellow, 5-6 mm long; tubes short (ca. 0.5 mm long), glabrous; throats 4.5-5.5 mm long, ca. 3 mm wide,

markedly pubescent above with septate, yellowish, or less often, purplish, hairs. **Stamens**, 5, their appendages ovate-lanceolate, eglandular. **Style branches** linear, purple, their appendages linear-lanceolate, the latter ca. 2 mm long. **Achenes** ca 1.5 mm long, densely white-pubescent with stiffly ascending hairs, the pappus of ca. 12, white-membranous, lanceolate scales ca. 5 mm long, their midveins extended into awns, 1-2 mm long.

TYPE: MEXICO. COAHUILA: 82 road mi SW of Cuatro Cienegas, ca. “7 mi S of turnoff for Los Delicias [sic], south of pass between Sa. Delicias and Sa. de Candelaria in pure gypsum w-facing slopes of Sierra la Candelaria at base camp of a Strontium mine ... 1.4 mi NE of Hwy 30,” 4040 ft, 12 Nov 2002, *James Henrickson 23199* (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: COAHUILA: “12 km NNE of Las Margaritas on the easternmost ridge of the Sierra de las Margaritas,” 1300-1400 m, 26 33 30 N, 102 51 30 W, 24 Sep 1972, *Chiang et al. 9508* (LL); “ca 32 (air) miles NE of Tlahualilo, in the NW portion of the Sierra de las Delicias, in the First Canyon S of the Puerto de las Sardines; on limestone [sic, presumably gypsum]; frequent perennial,” 4400 ft, 26 20 N, 103 06 W, 9 Aug 1973, *Henrickson 12208* (LL); (same locality and date as the type): *Henrickson 23199B* (TEX); *Henrickson 23200* (TEX).

All of the above specimens are very similar; however, Henrickson noted on the label of his collection *23199B* (presumably an isotype) that “of 42 plants, one small cluster of plants had red at tip of disk corolla lobes,” this observation presumably due to the purplish hairs alluded to in the above description.

Gaillardia candelaria superficially resembles **G. multiceps** of the southwestern U.S.A. It is immediately distinguished from that taxon by its elongate peduncles and subscapose habit (which superficially resemble that of the widespread, common *Tetrandeum scaposa*) and narrower, more enrolled, leaves. It also possesses longer, more linear, style-branch appendages.

Apparently, Henrickson originally intended to treat **G. candelaria** as a new variety (*G. multiceps* var. *candelaria*), to judge from his tentative identifications on the labels concerned. My examination of the material suggests specific status, although I concede that one’s species concept can be expanded to exceptional limits, often depending upon whim and circumstance.

The distributions of **Gaillardia** gypsophiles in north central Mexico are shown in Fig. 2.

ACKNOWLEDGEMENTS

I am deeply indebted to George Hinton and Mike Moore for reviewing the paper, and their exceptional input, and to my consummate editor, Jana Kos, for proof reading the manuscript. Figures 3 and 4 were provided by George Hinton; Figure 5 by M. Moore.

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Fig. 1. *Gaillardia c. var. mikemoorei* (Holotype: MEXU; isotypes: GBH, TEX).

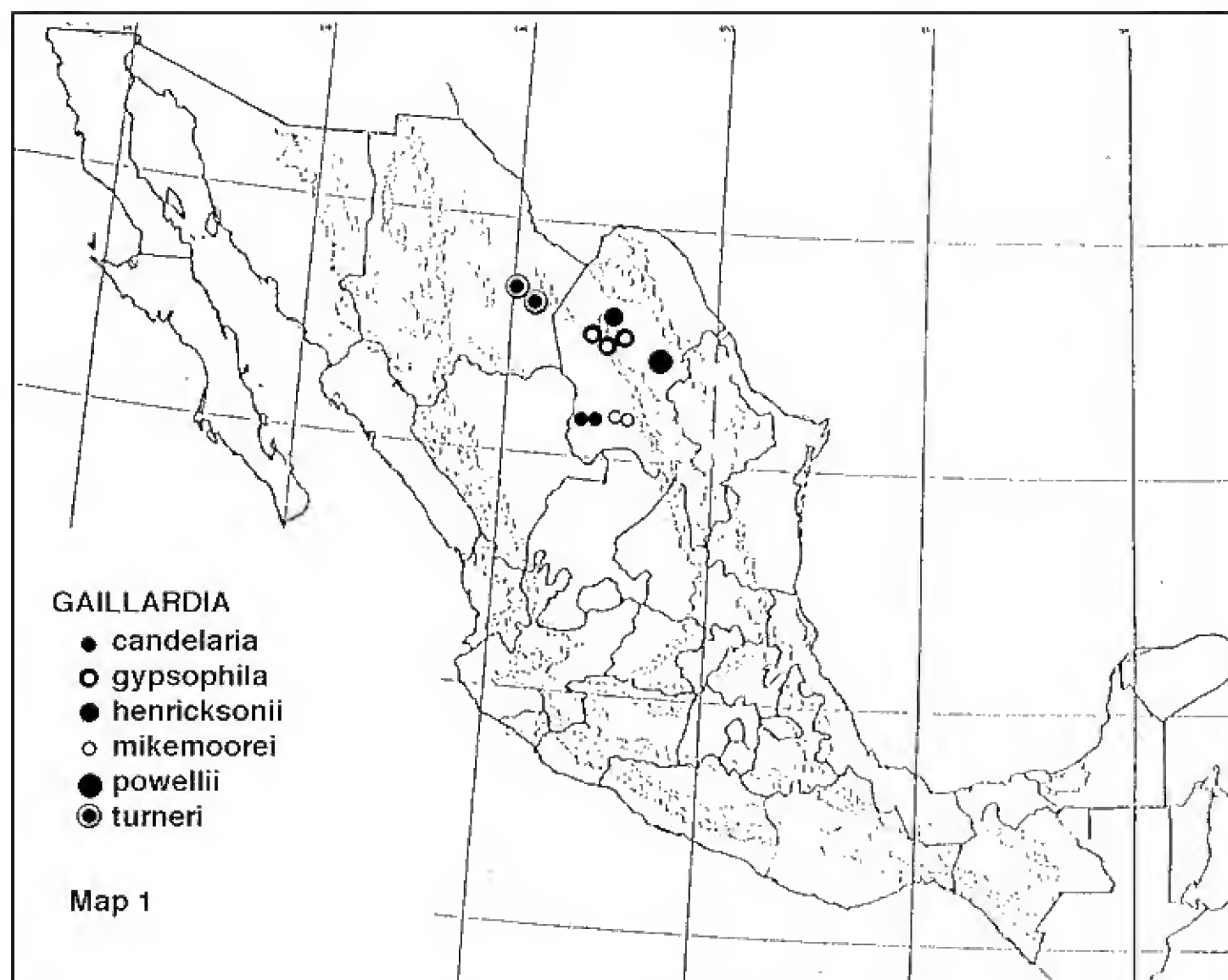


Fig. 2. Distribution of *Gaillardia* gypsophiles in Mexico.



Fig. 3. *Gaillardia c. mikemoorei* in the field.



Fig. 4. Type locality of *G. c. mikemoorei*.



Fig. 5. Mike Moore, gyp site, with Guadalupe Mts. of Texas in background.

Germination of achenes of *Chaptalia texana* Greene (Silverpuff) a perennial Asteraceae

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ABSTRACT

Some of the germination requirements of *Chaptalia texana* Greene (Silverpuff), a perennial Asteraceae, were examined. *Chaptalia texana* is found in central and western Texas, in southern New Mexico, northern, central and southern Mexico. In central Texas it seems to occur in savanna communities, below the canopy of *Juniperus-Quercus* (juniper-oak) mottes or woodlands, but infrequently in adjacent grasslands. Mostly population densities were low, and it appears to have an aggregated or clumped distribution with a high density under some woodland canopies and low density away from the canopies. Flowering seems to occur year round, though mainly when temperature and rainfall are moderate. Achenes of *C. texana* were slightly dormant, but started to germinate seven days after initial collection with 50% germination in 7.5 days. Initial germination immediately after collection, pappus removal and 16 days of incubation at 25°C in low light was 100±0% ($\bar{x} \pm SD$) by day 12. Germination decreased to 82±4% with dry storage of 12 weeks at 25°C and was expected to decrease to approximately 49% after 36 weeks of dry storage at 25°C. Achenes with pappus intact and incubated at 25°C had 95±2% germination. However, this was not significantly different from achenes with pappus removed. *Chaptalia texana* had very little or very slight innate dormancy and a majority of achenes could survive at moderate temperatures and moisture for over six months. Achenes would probably not germinate in summer or winter because of enforced dormancy at high summer or low winter temperatures and low soil moisture in summer. Published on-line www.phytologia.org *Phytologia* 95(4): 255-263 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Germination, dormancy, achenes, seeds, *Chaptalia*, silverpuff, perennial, Asteraceae

When and where a species will grow, flower and produce seeds is dependent on the habitat and a particular set of environmental conditions typical of that habitat. The factors would usually include specific temperature, rainfall, photoperiod, and probably the presence or absence of neighbors (Begon et al. 2006; Smith and Smith 2012). Seed germination and growth, especially the timing of germination and early growth seem to be very important to understanding the adaptations of a species to its environment and where it will be found (Baskin et al. 1995). Seeds of almost all flowering plants are initially dormant when shed from the parent (Begon et al. 2006). Regardless, seeds of every species respond to a characteristic set of environmental conditions. Conditions required for germination should be followed by another set of conditions favorable to seedling survival, growth, and reproduction (Fenner 1985). These germination and growth requirements probably include a specific temperature range, specific light levels, photoperiods, salt levels, nutrients and nutrient levels, possible seed scarification, red/far-red ratios, or some combination of factors (Mayer and Poljakoff-Mayber 1989; Bewley and Black 1994).

Chaptalia texana Greene or silverpuff (sunflower or Asteraceae family, Fig. 1) apparently can flower year-round in some parts of its range (Nesom 1995), but winter frosts and hot-dry summer conditions typically suppress or stop its growth and flowering during part of the year along the southern edge of the Edwards Plateau region of central Texas (personal observation). *Chaptalia* is a genus of about 56 species, with two species in the southern United States, and the remainder in Mexico, Central and much of South America (Correll and Johnson 1979; Nesom 1995). *Chaptalia texana* is found in central and western Texas, in southern New Mexico, northern, central and southern Mexico (Nesom 1995;

USDA 2009). In central Texas it occurs in savanna communities, below the canopy of *Juniperus-Quercus* (juniper-oak) motts or woodlands, but not in the adjacent grasslands (Nesom 1995; Van Auken and Bush 2013). *Chaptalia texana* is an herbaceous perennial and grows as a rosette in the woodland, and mostly not the grassland phase of some of these central Texas savanna communities (Correll and Johnston, 1979;



Figure 1. Photographs of *Chaptalia texana* plants with the rosette growth form below a *Juniperus-Quercus* woodland in central Texas. “A” shows a flower bud on a long scape (upper-left-center) and two inverted white tomentose leaves (lower left), “B” shows an inflorescence in seed on an elongated scape. Photographs were taken by Kelly Jo Stephens.

Enquist 1987; USDA 2009). It seems to be a secondary species found below the canopy of some of these woodlands. Many of the species of *Chaptalia* appear to be associated with the canopy of various species of *Quercus*, *Pinus*, *Juniperus* or some combination of them usually in a woodland, savanna or edge community (Nesom 1995).

Previous studies suggested that *C. texana* is a shade adapted species that can grow and carry out photosynthesis in the shade below a woodland canopy (Van Auken and Bush 2013). The physiological differences between plants of full sun compared to those found in shady habitats are fairly well known and have been used to delineate species habitat preferences (Begon et al. 2006; Valladares and Niinemets 2008). *Chaptalia texana* has maximum photosynthetic rates higher than typical understory plants at high light levels (Hull 2002). This is an interesting conundrum because photosynthetic rates suggest that this species should be found in grasslands and results do not explain why it is not found in grasslands of these central Texas or other savannas. However, gas exchange rates or growth rates of any of the other species of *Chaptalia* have not been identified. In addition, no ecological studies of the successional status, disturbance requirements, densities or resource requirements of this species have been identified. Thus, its ecological niche and factors affecting its distribution are not well recognized.

When a species is found in a given habitat, it is because that species can tolerate or requires the environmental conditions present in that habitat. However, sorting out the characteristics or factors that determine why a species is present or dominant where it is found and not in other habitats is much more challenging (Begon et al. 2006). Various grasses do not grow with *C. texana* in the canopy understory and at the same time *C. texana* does not seem to grow with or occur with various grasses in open central Texas grasslands or savannas (Van Auken and Bush 2013). It seems that light levels would be the obvious factor controlling this distribution, yet there seem to be other factors.

Before some of the above can be studied or tested using controlled conditions, factors that seem to determine *C. texana*'s germination and early growth should be understood so seedlings might be available for ecological studies. This would allow transplant or manipulation studies to be carried out to understand specific requirements and niche characteristics of this species. Purposes of the experiments completed and reported here were to examine possible dormancy and some of the factors responsible for promoting germination of *C. texana*. In general, I hypothesized that *C. texana* would have minimal innate dormancy, but that temperature and storage could be important in breaking dormancy and promoting germination. I investigated germination of *C. texana* at one temperature and relatively short storage times, as well as effects of the presence or removal its pappus.

MATERIALS AND METHODS

To examine seed dormancy in *Chaptalia texana* (Fig. 1), the storage temperature and time required to promote germination or break dormancy was examined. Mature *C. texana* achenes (seeds) were collected in October and early December 2012 in San Antonio, Texas, USA (Bexar County, 98°36'W, 29°37'N). Collected achenes or seeds were placed in plastic bags at room temperature (25 °C). Immediately after achenes were collected, all that were to be placed in an experiment were visually inspected for fullness. Wrinkled or unfilled achenes were discarded. Some full achenes had their pappus removed. Initial germination was examined without any temperature or storage treatment. The experiment was repeated twice, once in October and a second time in early December. There were three replicates in each experiment and the pappus of all achenes was initially removed by breaking at the attachment point to the achene. Each replicate included 20 achenes placed on one, 7.0 cm diameter, Whatman® number one qualitative filter paper, that was placed in a 100 mm diameter X 15 mm deep disposable polystyrene petri dish. The filter paper was moistened with 5 ml of deionized water, and then the petri dishes were closed and placed in 17.8 cm X 20.3 cm plastic bags to retard moisture loss. They were kept at 25°C in low light ($250 \mu\text{M}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, Li-Cor® LI-188 integrating quantum sensor) for 15-18 days depending on the experiment and the germination response. All petri plates were examined every day and any achenes that had germinated (radical emerged at least 1 mm) were counted, the number was recorded, and germinated achenes were removed, placed in fiber pots with soil or discarded.

All stored achenes were kept dry and at a temperature of 25°C with some stored for three months. Initially and after 2.5 weeks and three months storage treatment time, germination was tested as above with the pappus removed. An additional study was completed with achenes with pappus attached. Each replicate was 20 achenes placed on one, dry, 7.0 cm diameter, Whatman® number one qualitative filter paper in a 100 mm diameter X 15 mm deep disposable polystyrene petri dish as above. This experiment was started 2.5 weeks after the achenes were collected. There were three replicates of 20 achenes each with the pappus intact and three with the pappus removed. Germination was observed and conditions were as indicated above and the experiment was completed in 16-18 days.

Data was analyzed using the SAS JUMPRO statistical package (SAS-Institute 2011). The experiments were analyzed as one-way ANOVAs with days of germination or time (storage time) as the independent variables. If significant differences between mean number of germinations and the

independent variables were detected, a Tukey-Kramer HSD multiple comparison test was employed for pair wise comparisons among individual treatments. All germination data was arc-sine transformed prior to analysis in order to normalize the distributions (Kleinbaum et al. 1988).

RESULTS

Initial dormancy of *Chaptalia texana* achenes was examined by testing their ability to germinate as soon as they were harvested. Initial mean germination was $100 \pm 0\%$ ($\bar{x} \pm \text{SD}$) with 7.5 days of germination treatment on wet filter paper in petri plates at 25°C in low light required to reach the mean T_{50} (number of days to 50% of final germination) (Fig. 2). Achene germination started on the seventh day

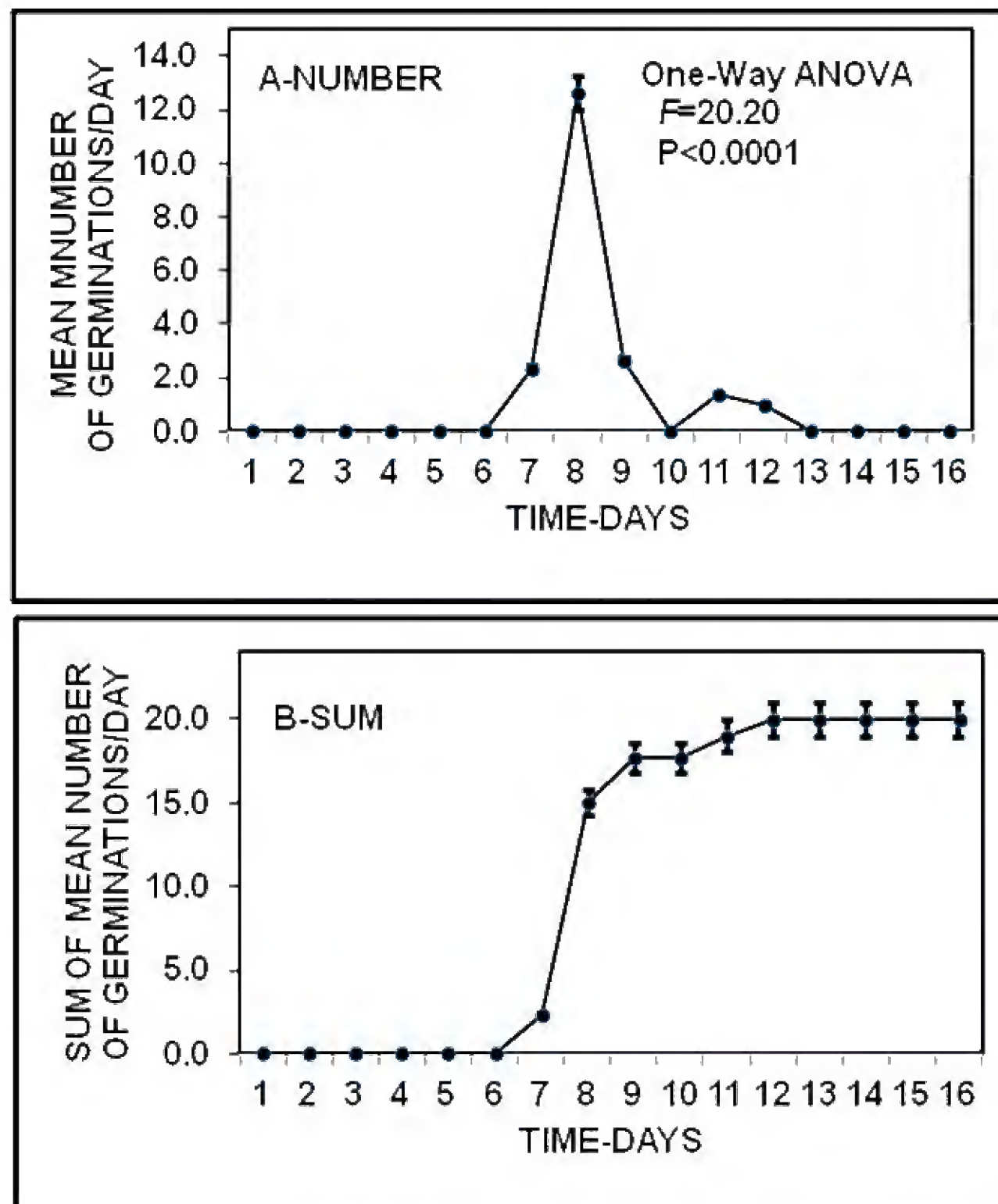


Figure 2. Mean number of achenes germinating per day (A) and the running mean of total germinations (B) for *Chaptalia texana* subjected to a germination test immediately after harvest. There were three replications with 20 achenes/replication. Conditions were 25°C and low light in petri plates. Final total was 100% (60/60 achenes) germinating. Germination started on day seven with a maximum on day eight. T_{50} or the number of days to 50% of final germination was 7.5 days. Achenes were harvested on 12-3-2012 and the germination test was started the same day. The one-way ANOVA was significant, but the number of germinations on day eight was the only day that the number of germinations was significantly different from all the others (Tukey- Kramer HSD test, $P < 0.05$).

of treatment and all achenes germinated by the 12th day of treatment. The one-way ANOVA was significant, but the number of germinations on day eight was the only day that the number of germinations was significantly different from all the others, which were not different from each other (Tukey-Kramer HSD test, $P < 0.05$). The presence or absence of the pappus on the achenes did not change the total number of germinations (one-way ANOVA, $F = 6.0$, $P = 0.07$) (Fig. 3). If the pappus was attached, final germination was $95 \pm 5\%$ and started on day 10 and was at a maximum the same day with slight declines on the 11th and 12th days. The mean T_{50} was 10.5 days (Fig. 3A). If the

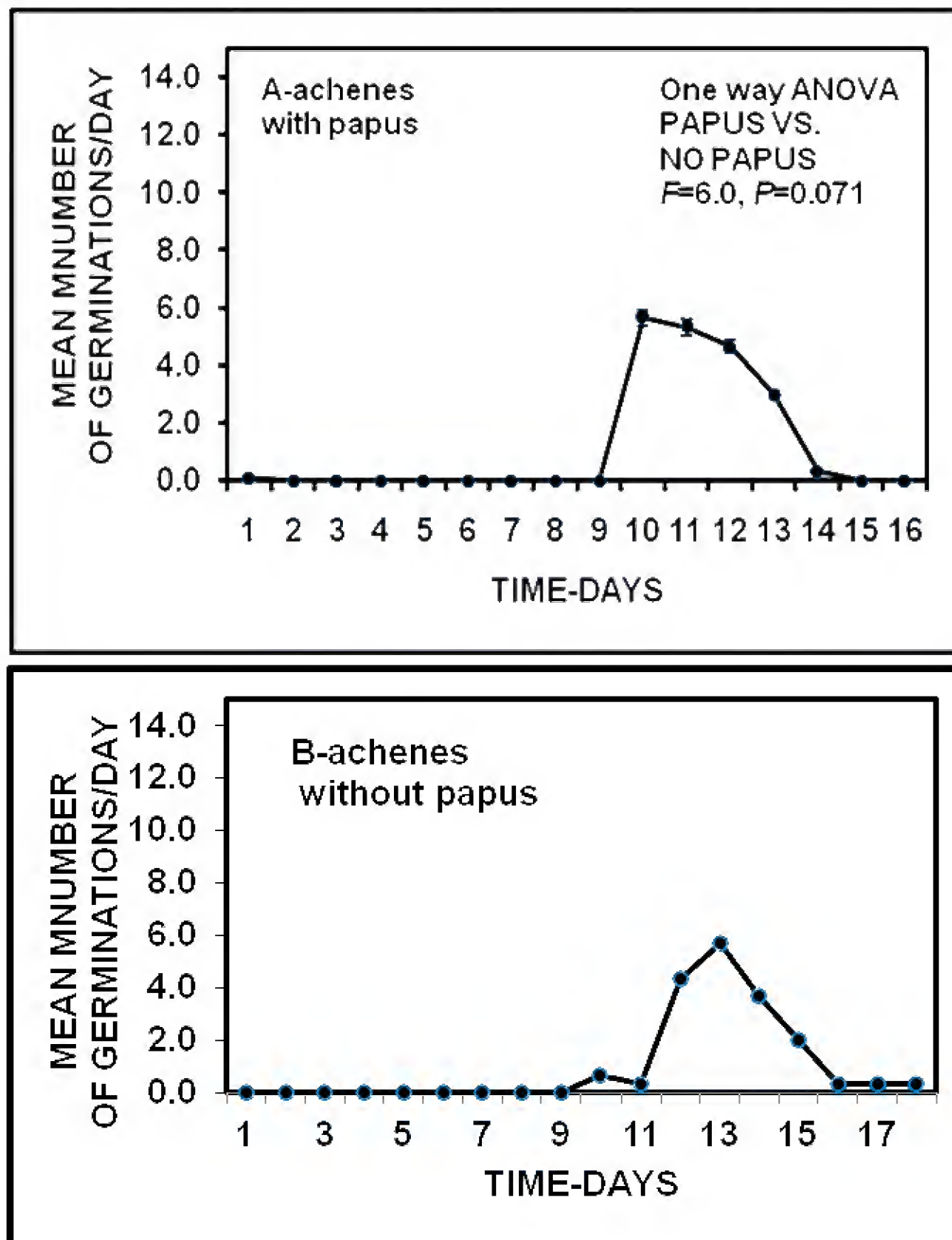


Figure 3. Mean number of achenes germinating for *Chaptalia texana* per day with pappus intact (A) or the pappus removed (B). There was no significant difference in total number of germinations between the two treatments (one-way ANOVA). There were three replications/treatment with 20 achenes/replication. The germination test was started 2.5 weeks after harvest. Conditions were 25°C and low light in petri plates.

pappus was removed, final germination was $88 \pm 5\%$. Germination started on day 10 with a maximum on day 13. The mean T_{50} was 12 days (Fig. 3B). With dry storage at 25°C and in low light, germination dropped to $88 \pm 5\%$ in two and one half weeks and then to $82 \pm 4\%$ in 12 weeks (Fig. 4). Simple linear

projections suggest that after 36 weeks of dry storage in low light that germination of *C. texana* achenes would be approximately 49%.

DISCUSSION

Seeds of flowering plants usually show some degree of dormancy and that includes members of the family Asteraceae (Begon et al. 2006). Non-dormant seeds would appear to be an exception. The beginning of dormancy occurs while the seeds are still attached to the parent when metabolic activity of the seeds dramatically slows. Germination of the seed and breaking of dormancy is critical to a plant's reproductive success, especially in unpredictable or fluctuating environments (Baskin et al. 1995). Breaking dormancy and thus germination for a given species would be in response to a characteristic set of environmental conditions typical of their habitat and could include specific temperatures, light levels, photoperiods, salt levels, nutrients, scarification, red/far-red ratios, or combination of these factors (Harper 1977; Taiz and Zeiger 1998; Smith and Smith 2012). Once seeds germinate, the subsequent environmental conditions should be favorable to seedling survival, growth, and reproduction (Fenner 1985).

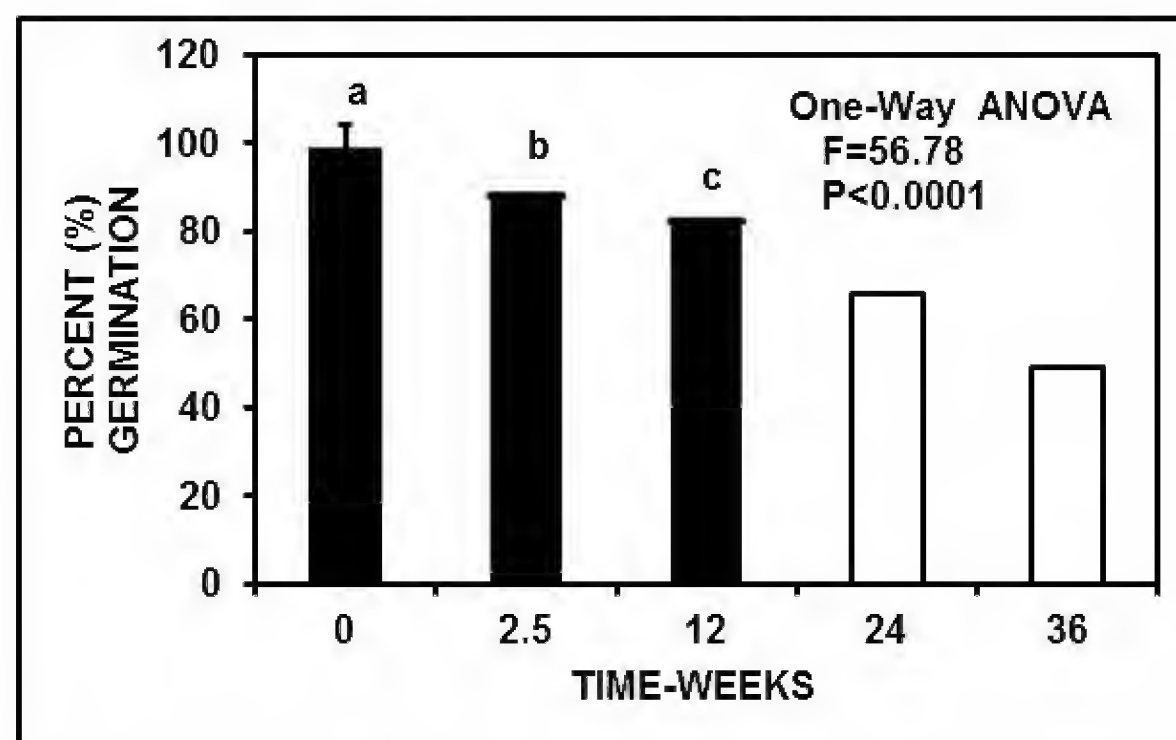


Figure 4. Mean percent germination of *Chaptalia texana* achenes after 0, 2.5, and 12 weeks of dry storage at 25°C and low light. Solid black bars are actual measurements and white bars are linear projections to 24 and 36 weeks. The line at the top of the zero week bar is one standard deviation of the mean. The percent of achenes germinating in the different storage times was significant (one-way ANOVA) and the different letters at the top of the bars indicate significant differences between storage treatments (Tukey Kramer-HSD test, $P<0.05$).

Seeds are dormant when they fail to germinate in spite of the presence of environmental conditions necessary for germination (Taiz and Zeiger 1998). There are three general types of seed dormancy that include innate, induced, and enforced dormancy (Nikolaeva 1977; Mayer and Poljakoff-Mayber 1989; Bewley and Black 1994; Taiz and Zeiger 1998; Begon et al. 2006). Seeds produced during the current growth year that fail to germinate under normally favorable growth conditions are innately dormant. These innately dormant seeds have an inhibitory mechanism that must be overcome before germination will begin. The mechanisms could be mechanical, chemical, or morphological and could include seed coat impermeability, light or temperature sensitivities, chemical inhibitors, or after-ripening requirements (Nikolaeva 1977; Mayer and Poljakoff-Mayber 1989; Bewley and Black 1994; Taiz and Zeiger 1998; Begon et al. 2006). Non-dormant seeds could be induced into dormancy when conditions that reduce seedling survival are present. The third type of dormancy is enforced dormancy and is caused by environmental conditions unfavorable to seedling growth and survival (Nikolaeva 1977; Mayer and

Poljakoff-Mayber 1989; Bewley and Black 1994). The three general types of dormancy may occur alone or in sequence, and possibly in the same seed or seed type.

Chaptalia texana achenes (seeds) demonstrated slight or little innate dormancy. Once collected and placed on wet filter paper with pappus removed at 25°C and low light, 100% of the achenes germinated. Germination started after seven days and all achenes germinated by day 12. Predicted 100% dormancy or 0% germination as soon as the seeds were mature and released from the parent (Begon et al. 2006) lasted seven days. However, most of this time could have been required for embryo and radical growth as well as escape from the achene coverings. Thus, innate dormancy in *C. texana* appears to be slight or minimal and easily overcome. Results were similar for a number of other south-central Texas Asteraceae (Baskin et al. 1998). Innate dormancy, when present, was broken with a short period of cold stratification (Baskin et al. 1992; Baskin et al. 1995; Baskin et al. 1998).

The presence of a pappus on achenes of *C. texana* had little effect on germination. The pappus is important for dispersal, but apparently not for germination success. However, as storage time increased, germination success decreased (Fig. 4). Estimated germination after 36 weeks was 49%. The achenes that did not germinate appeared to lose viability and were covered with fungal growth. Loss of viability could have been due to moisture loss by the stored achenes, but this was not examined.

Chaptalia texana along the southern edge of the Edwards Plateau region of central Texas appear to flower year round and mature achenes would be dispersed accordingly (Enquist 1987; Nesom 1995). June, July, and August as well as December and January in central Texas are times of environmental extremes. Monthly high temperature and precipitation means (NCDC 2000) in the San Antonio area for June-August are 33-35°C and 9.4-5.5 cm respectively, with some years having no rainfall in one or more of these months. In addition, mean high minimums are approximately 26°C. Thus, maturity and dispersal of *C. texana* achenes in summer, if at all, occurs under conditions of high temperatures and low rainfall. Germination of *C. texana* achenes during south Texas summers is probably low and moisture limited. With low rainfall conditions in summer, most achenes would likely not germinate due to enforced dormancy initiated by moisture limitations.

Germination results suggest that with sufficient moisture during summer in south Texas all *C. texana* achenes could germinate. However, because of potentially high drought induced seedling mortality in summer, those achenes that germinated immediately following dispersal would probably not survive. If substantial yet infrequent summer rainfall events triggered those achenes to germinate, the subsequent high temperatures, inconsistent rainfall and soil moisture would probably reduce seedling growth and survival close to zero. With flowering in fall and achene dispersal at that time, survival would be greater.

Decreased temperatures and increased rainfall in early fall would insure that the shallow central Texas soils (Taylor et al. 1966) would remain moist for a longer period of time (Fay et al. 2003; Wayne and Van Auken 2004) and that would probably increase *C. texana* seedling survival and plant growth. Therefore, the greatest *C. texana* seedling survival probably occurs in those years in which rainfall events in June, July, and August are infrequent and small, enforcing dormancy upon all *C. texana* achenes until temperatures are cooler and rainfall more frequent in September and October (NCDC 2000).

Chaptalia texana's usual growth form is a basal rosette. This is a growth form used by many winter annuals to get an early start in the spring when temperatures increase and photoperiods get longer (Fenner 1985; Baskin et al. 1992; Bewley and Black 1994; Baskin et al. 1998). However, I know of no studies concerning growth and survival of *C. texana* seedlings or mature plants as basal rosettes. Their survival may have something to do with poor detection resulting in little consumption by large herbivores, but this is undocumented at this time.

Seedling success of *C. texana* is not assured after achenes germinate. The highest probability occurs after achene germination when a set of conditions follow that are favorable for seedling survival, growth, and reproduction (Fenner 1985). Nevertheless, beyond abiotic factors, various positive, negative, or neutral biotic factors (facilitation or competition), are often cited as playing major roles in determining species success, community composition and structure (Harper 1977; Connell 1983; Callaway 1995; Bertness and Leonard 1997; Callaway and Walker 1997; Holmgren et al. 1997; Bush and Van Auken 2010; Smith and Smith 2012).

Competition may be a major factor determining a plant or species establishment success, and then determining where it is found in the future (Harper 1977; Connell 1983; Schoener 1983; Smith and Smith 2012). Competition from associated grassland species or inherent lack of competitive ability may cause *C. texana*'s apparent restriction to juniper-oak canopies (Van Auken and Bush 2013). Nonetheless, *C. texana* does not appear to be a true understory or shade species. It has high photosynthetic rates when exposed to high light levels, but it seems to be restricted to areas below the canopy in low light because it cannot compete with the grasses growing in the open grassland. There seems to be another environmental factor or a combination of factors that limit the growth of *C. texana* to shaded understory habitats and prevent it from growing in open grassland habitats or disturbed grassland habitats. This is an interesting conundrum because photosynthetic rates suggest that this species should be found in grasslands and open savannas and results do not explain why it is not found in these habitats. A similar pattern has been reported for a native mustard (Crucifer), but the restriction is caused by herbivory (Leonard and Van Auken 2013). These theories or ideas have never been tested or demonstrated for *C. texana*. It appears that soil water may be limiting the growth of *C. texana* in grasslands because of water use by potentially more drought tolerant grasses, which keeps *C. texana* restricted to canopy habitats where the grasses cannot grow or compete because of low light levels and their high light requirements.

ACKNOWLEDGEMENTS

Specific thanks for reading an earlier draft of this manuscript and making many helpful comments and corrections go to Donna Taylor, Environmental Research Scientist, Cibolo Nature Center, Borne, Texas; Wendy Leonard, City of San Antonio Parks and Recreation Natural Areas, San Antonio, Texas; and Jerrett W. Nunneley, Research Associate, Texas Biomedical Research Institute, San Antonio, Texas.

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Intraspecific variation in *Aldama dentata* (Asteraceae: Heliantheae)

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ABSTRACT

The commonly encountered Mexican and Central American species, *Aldama dentata*, considered by most previous workers as having but two varieties, is now enriched with a third such taxon, **A. dentata** var. **salvatierra**, B.L. Turner, **var. nov.** It is known only from the state of Guanajuato, Mexico. Published on-line www.phytologia.org *Phytologia* 95(4): 264-268 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Asteraceae, Heliantheae, *Aldama*, *A. dentata*, Mexico, Guanajuato

The taxonomy of **Aldama dentata** has long been in dispute, as indicated by its considerable synonymy, some workers accepting the genus as monotypic (Feddemma 1971, Rzedowski and Rzedowski 2008); others positioned it variously, as indicated by its synonymy. Recent DNA studies by Schilling and Panero (2002; 2011) have shown that the genus should be expanded to include numerous taxa previously placed in *Viguiera* (sensu lati).

Feddemma (1971) treated **Aldama** as monotypic; he recognized **A. dentata** as having but two varieties, and I follow his treatment, except for the addition of a third variety, described below.

Key to varieties

1. Involucres 9-12 mm high; ligules 1.5-2.0 cm long;
inner involucral bracts acute, pubescent with bullate-
based, strigose, hairs, the latter 1-2 mm long; ne Mic
and adjacent Jalvar. **zamorensis**
1. Involucres 5-8 mm high; ligules mostly 0.6-1.5 mm
long; inner involucral bracts mostly obtuse, pubescent
with shorter hairs, these not especially bullate, widespread ...(2)
2. Annual or perennial, much branched, suffruticose herbs or shrubs
mostly 0.3-3.0 m high; ultimate peduncles mostly 3-10 cm long; widespread..... var. **dentata**
2. Annual, stiffly erect, mostly unbranched, herbs 30-50 cm high; ultimate
peduncles, densely pilose, 1-2 cm long; Gua..... var. **salvatierra**

ALDAMA DENTATA La Llave & Lex., Nov. Veg. Descr. 14. 1824.

Perennial, or seemingly annual, herbs to 3 m high. Stems stiffly erect but often rooting at the lower nodes. Leaves mostly alternate, lanceolate, petiolate, entire to dentate. Heads, few to numerous, campanulate, radiate. Involucres, 2(3)-seriate, the bracts herbaceous and subequal, broadly ovate to elliptical, usually streaked with black lines. Receptacles convex, paleate. Ray florets neutral, sterile, the ligules well developed, yellow. Disk florets yellow, mostly completely enveloped by the scarious, subtending, receptacular bracts, these sometimes becoming indurate and markedly crinkly toward the periphery of the head. Achenes flattened radially, black, striate, glabrous, epappose or nearly so. Chromosome number, n = 17 pairs.

var. **dentata***Gymnolomia acuminata* B. Rob. ex Blake*Gymnopsis dentata* La Llave & Lex.*Gymnopsis schiedeana* DC.*Sclerocarpus acuminatus* B. Rob.*Sclerocarpus dentatus* (La Llave & Lex.) Benth. & Hook.*Sclerocarpus elongatus* (Greenm.) Greenm.*Sclerocarpus kerberi* Tourn.*Sclerocarpus schideanus* (DC.) Benth. & Hook.

Sin, Tam, San, Que, Hid, Nay, Jal, Col, Gua, Mic, Mex, Mor, Pue, Ver, Tab, Gue, Oax, Cps, Guatemala and southwards, 100-2000 m; all seasons.

This is an extremely common, highly variable, taxon along the Gulf coastal slopes, being especially abundant in disturbed areas, and fallow fields of Ver and Oax. Shade-forms may occur side-by-side with sun-forms, the latter with appressed sparsely strigose stems, the former with much thicker vestiture and spreading hairs on longer leaves. Collections of this taxon from Nay, Jal and Mic tend to have outer disk florets with tangentially flattened achenes at maturity, their enclosing bracts becoming highly wrinkled and sclerified, the entire structure (i.e., bract and enclosed achene) often nearly as wide as high. These traits are, collectively, not found in specimens from eastern Mexico (*Sclerocarpus acuminatus*, of authors) and it is probable that future workers will recognize additional regional facies in this species; chromosome number, $n = 17$ pairs.

var. **salvatierra** B.L. Turner, var. nov. Fig. 1

Stiffly erect, unbranched, tap-rooted annual to 60 cm high. Stems (upper) moderately pubescent with upwardly appressed hairs, 0.5-1.0 mm long. Leaves (mid-stem), 5-7 cm long, 1.0-1.5 cm wide; petioles 2-6 mm long; blades linear lanceolate, markedly pubescent above and below, the margins entire, or nearly so. Capitulescence a terminal or axillary cymose panicle of (1)2-10 heads, the ultimate peduncles densely pubescent, 8-10 mm long. Heads (rays excluded), ca 6 mm high, 7 mm wide. Involucres, 4-5 mm high, campanulate; outer bracts ca 10 in 2 equal series, ovate to obovate, markedly pubescent. Receptacles hemispheric, ca 1 mm high, 2 mm across. Pales ca 2.5 mm long, enveloping the achene, their apices purplish-sclerose. Ray florets 5, neuter, sterile; ligules yellow, ca 5 mm long, 3-5 mm wide, Disc florets, numerous (40 plus); corollas yellow, 3-4 mm long; tubes ca 1.5 mm long; throats ca 2 mm long, sparingly pubescent with minute hairs; lobes ca 0.75 mm long. Anthers yellow. Achenes ca 2.5 mm long, obovate, glabrous, epappose.

TYPE: MEXICO. GUANAJUATO: Mpio. Salvatierra, "alrededores de la poblacion," 1800 m, 12 Oct 1985, Rzedowski 39142 (Holotype: TEX).

The stiffly erect, unbranched, habit, more densely pubescent foliage, and more numerous heads on shorter ultimate peduncles distinguish this novelty from the typical var. **dentata**. Rzedowski and Rzedowski (2008) treat the above type, and several additional collections from near the type locality, as part of their broad concept of **Aldama dentata**, a treatment that I accepted early on, and such might yet prove the better alternative, considering the exceptional populational variation found in the species.

The appellation is derived from the Mpio. Salvatierra.

var. **zamorensis** Feddema, Phytologia 21: 313. 1971.

Known only from the vicinity of Zamora, Mic, and adjacent Jal in fallow wet fields, 1600-1800 m; Aug.

Robust, rank, perennials with harsh, spreading, hispid hairs (1-3 mm long) on the stems, the outermost disk florets radially compressed at maturity, the enclosing bracts thin and only slightly crinkled, at most; chromosome number, $n = 17$ pairs.

This taxon appears to be quite distinct, and future workers should attempt to ascertain its biological status with more certainty. However, similar variation patterns to that noted in var. **zamorensis** are found in Oax and Cps (e.g., *Ton 1934*, LL) suggesting that very divergent local populations might be found almost anywhere within the range of the broadly distributed species, **A. dentata**.

Strangely, McVaugh (1987) did not accept the validity of var. **zamorensis**, in spite of the fact that it was proposed by one of his Academic children (Feddema) in 1971, the holotype at MICH. McVaugh did, however, cite his paper in which the name was published.

Distributions of the several varieties are shown in **Fig. 2**.

ACKNOWLEDGEMENTS

My editorial assistant, Jana Kos, provided input, for which I am grateful. Distribution maps are based upon specimens on file at LL-TEX, and reports on the web and in the literature.

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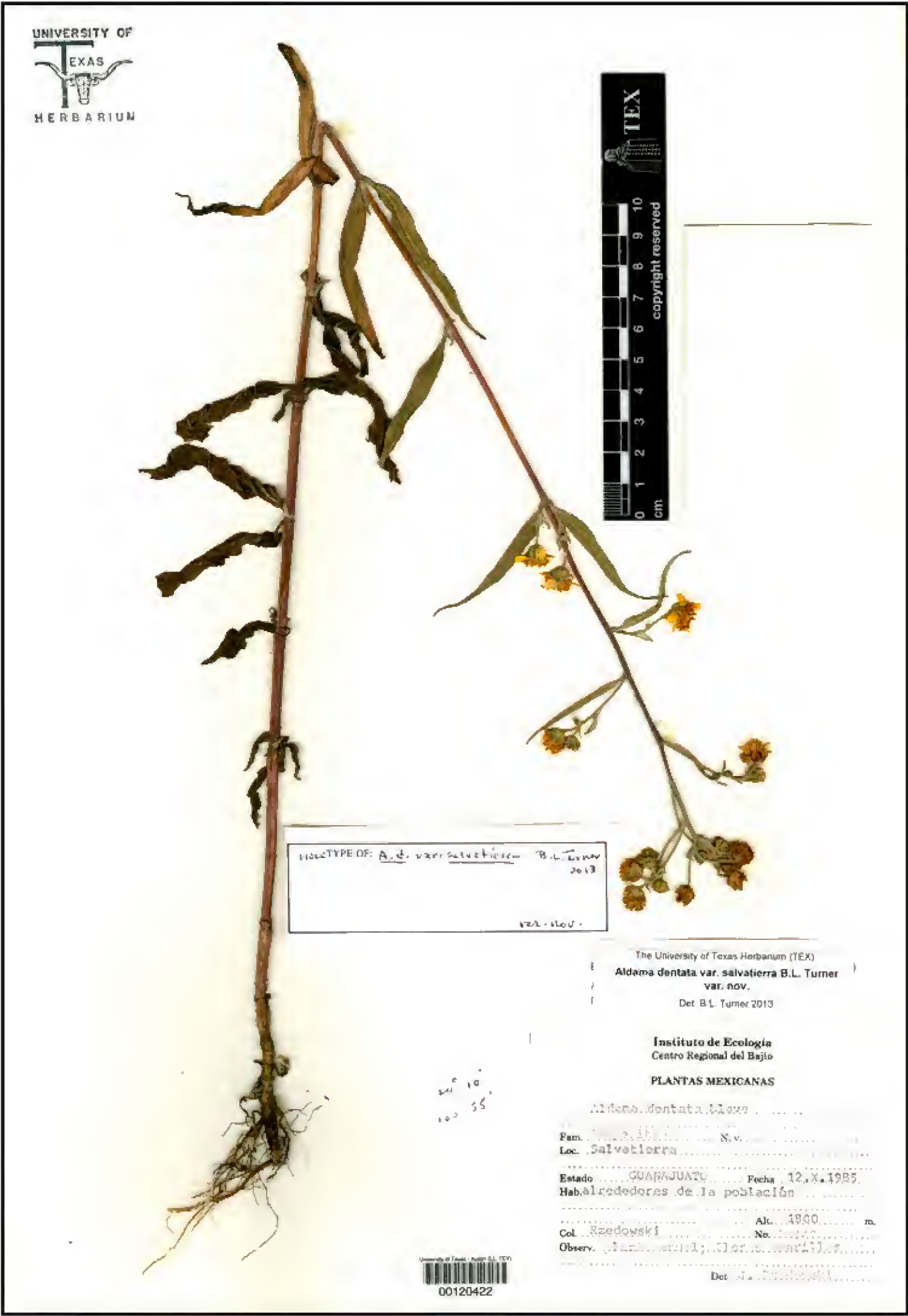
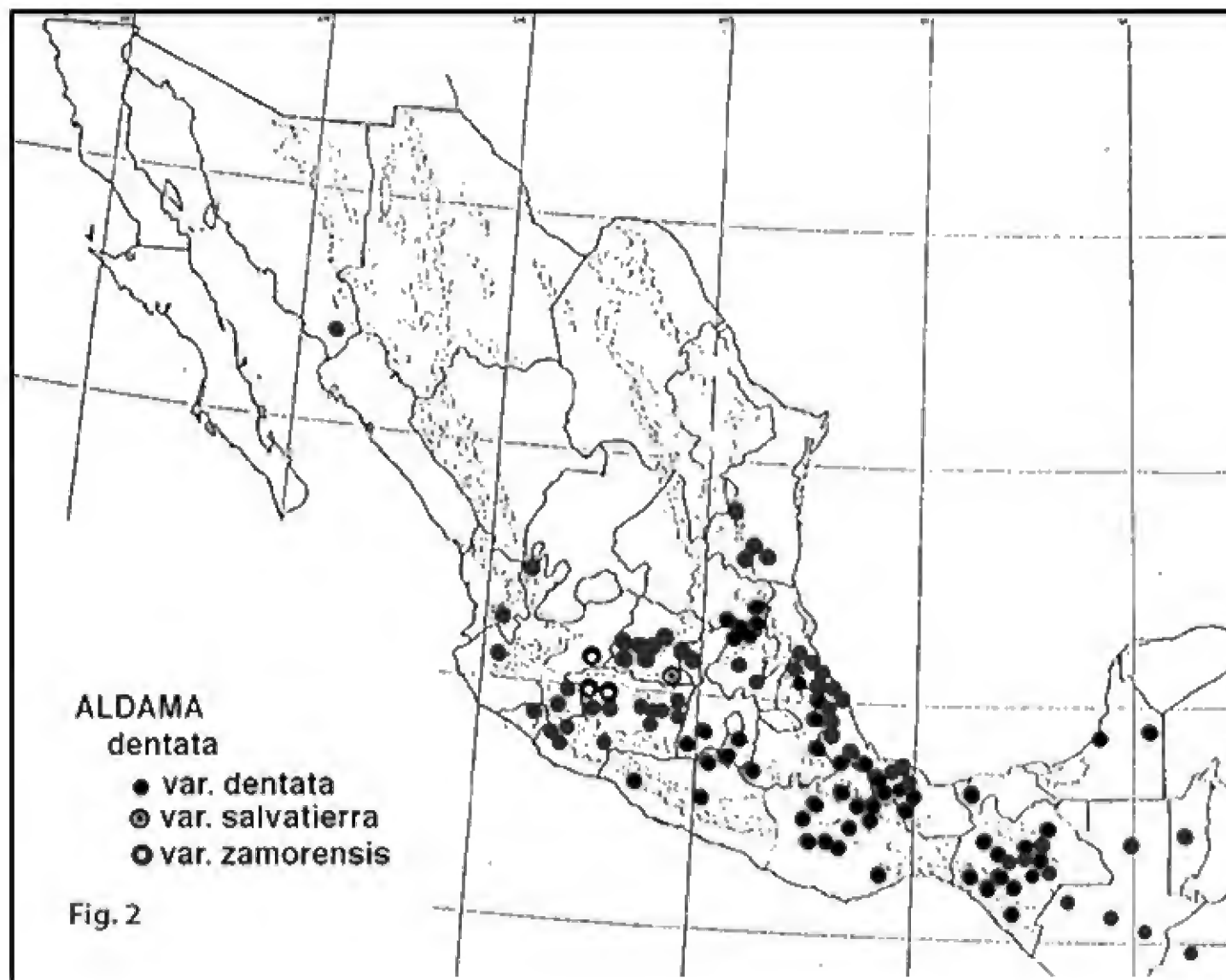


Fig. 1. Holotype of *Aldama dentata* var. *salvatierra*.



Geographic variation in the volatile leaf oils of *Juniperus procera* Hochst. ex. Endl.

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ABSTRACT

Comparisons of the leaf components for four populations of *J. procera* are reported. The major components of the oils are α -pinene (17.7 to 37.4%), δ -3-carene (8.1 - 28.7%), terpinolene (1.8 - 4.3%), elemol (1.4 - 6.2%), abietadiene (0.6 - 11.8%) and trans-totarol (1.3% - 11.0%). There appears to be a North - South cline of variation from Abha, Saudi Arabia to Ethiopia to Kijabe, Kenya (cf. -pinene, -3-carene, elemol, abietadiene and trans-totarol. However, the population at Thika does not fit the trend and is very unusual in all the major components. It was observed that a considerable number of trees were planted in the Thika area and perhaps the samples of 'native' trees were in fact obtained from cultivated trees. Published on-line: www.phytologia.org *Phytologia* 95(4):269-273 (Nov. 1, 2013).

ISSN 030319430

KEY WORDS: *Juniperus procera*, geographic variation, leaf oils, terpenes.

Juniperus procera Hochst. ex. Endl. is the only juniper that grows naturally in both the northern and southern hemispheres; all other *Juniperus* species are confined to the northern hemisphere (Adams, 2011). *Juniperus procera* consists of two major populations: in Saudi Arabia/ Yemen and the high mountains of east Africa (Fig. 1). The species is thought to have originated from *J. excelsa*, or an ancestor that migrated southward into the Rift Mountains of east Africa (Adams, Demeke and Abulfatih, 1993).

The volatile leaf oils have been reported upon, and literature reviewed by Adams (1990). The purpose of this paper is to present an updated analyses of the oils and geographic variation in east Africa and Saudi Arabia.

MATERIALS AND METHODS

Plant material - *J. excelsa*: Adams 13193 (9433-9435), Eskisehir, Turkey, *J. procera*: Adams 6190-6193, ex. H. A. Abulfatih, Abha, Saudi Arabia, ca. 18° 13' N, 42° 30' E, 7300 ft., Adams 6184-6188, near Addis Alem, Ethiopia, 40 km west of Addis Ababa on road to Guder, 2400 m, ca. 9° 02' N, 38° 23' E, 2400m; Adams 5333-5335, near Thika, 38 km nw of Nairobi, Kenya, ca. 1° 02' N, 37° 03' E, 2170m; Adams 6007-6009, Kijabe, near Rift Valley Academy, ca. 0° 56' N, 36° 36' E, 7300 ft. Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

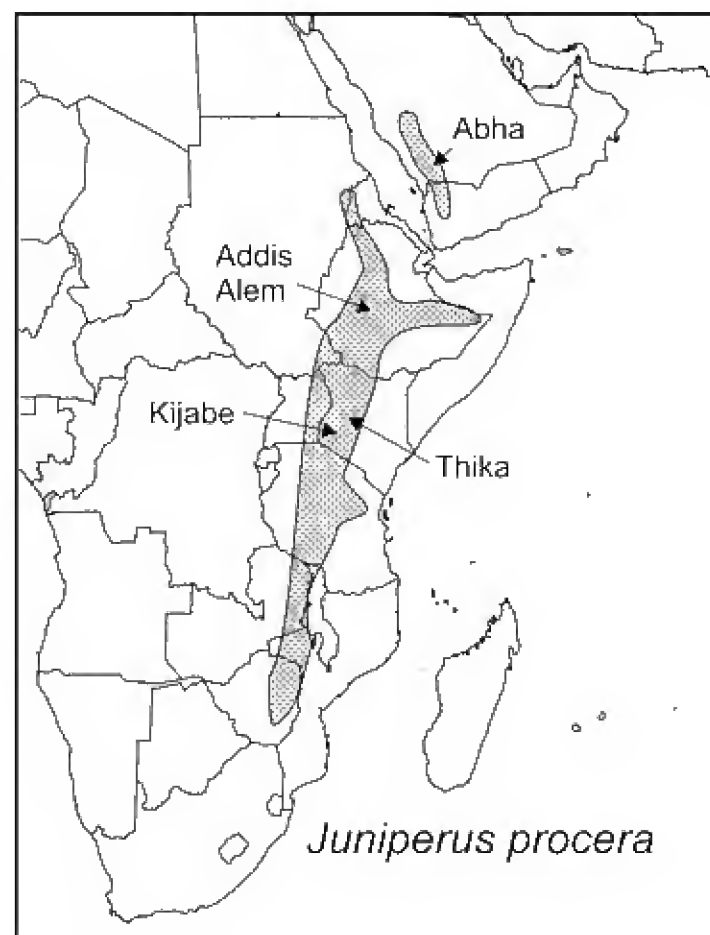


Fig. 1. Distribution of *J. procera* with populations sampled.

Fresh or air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time

1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

Comparisons of the leaf components for the four populations are given in Table 1. The major components of the oils are α -pinene (17.7 to 37.4%), δ -3-carene (8.1 - 28.7%), terpinolene (1.8 - 4.3%), elemol (1.4 - 6.2%), abietadiene (0.6 - 11.8%) and trans-totarol (1.3% - 11.0%). There appears to be a North - South cline of variation from Abha, Saudi Arabia to Ethiopia to Kijabe, Kenya (cf. -pinene, -3-carene, elemol, abietadiene and trans-totarol (Table 1). However, the population at Thika does not fit the trend and is very unusual in all the major components (Table 1). It was observed that a considerable number of trees were planted in the Thika area and perhaps my samples of 'native' trees were in fact cultivated. The seed source of the trees at Thika is not known.

The presence of cedrol (very common in the related, *J. excelsa* oil, see Table 1) is only 0.4% in the Abha samples and missing or just a trace in all the other samples (Table 1). It is tempting to speculate that the Abha population harbors some genes from *J. excelsa* from ancient hybridization with *J. excelsa*. Additional research is needed to support such conjecture.

It is also noteworthy that *J. procera* has been the source of East African cedarwood oil in the past (Adams, 1991). The heartwood oil is reported to contain 41.8% α -cedrene and 41.8% cedrol (Pettersson and Runeberg, 1961). But, as in the case with most *Juniperus* species, the leaf oil of *J. procera* contains an entirely different set of terpenoids than found in the heartwood oil. The heartwood oil components (cf. α -cedrene β -cedrene, thujopsene, cuparene, cedrol, widdrol, etc.) are nearly all absent in *J. procera* leaf oil, in contrast to *J. excelsa* (Table 1), *J. foetidissima*, *J. polycarpos*, *J. seravschanica* and *J. turcomanica* whose leaf oils contain large amounts of cedrol, etc. (Adams 2011).

ACKNOWLEDGEMENTS

Thanks to Billie Turner for review. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Table 1. Leaf essential oils for populations of *J. procera*. The leaf oil of *J. excelsa*, Turkey is added as a comparison. Components that tend to separate the *J. procera* populations are highlighted in boldface.

KI	Compound	Abha, S. A.	Addis Alem	Kijabe, Kenya	Thika, Kenya	<i>excelsa</i> Turkey
921	tricyclene	t	t	t	t	0.2
924	α -thujene	t	t	t	t	t
932	α-pinene	21.2	32.6	37.4	17.7	41.7
945	α -fenchene	1.0	1.1	0.8	0.2	0.3
946	camphene	0.1	0.2	0.3	0.2	0.2
961	verbenene	t	0.1	0.1	t	t
969	sabinene	t	t	t	0.7	0.1
974	1-octen-3-ol	0.2	0.3	0.8	1.2	-
974	β -pinene	2.1	3.1	3.7	2.2	0.7
988	myrcene	3.1	3.1	3.9	2.3	1.2
1002	α -phellandrene	t	t	t	t	0.1
1008	δ-3-carene	28.7	28.6	21.6	8.1	5.3
1014	α -terpinene	t	t	t	t	0.1
1020	p-cymene	0.3	0.2	0.2	t	0.6
1024	limonene	0.8	0.5	t	0.8	1.2
1025	β -phellandrene	1.5	1.4	2.3	0.8	0.9
1044	(E)- β -ocimene	t	t	t	t	t
1054	γ -terpinene	0.2	t	0.1	0.2	0.5
1086	terpinolene	4.3	3.2	2.3	1.8	1.1
1095	linalool	1.1	0.6	0.3	1.7	-
1108	p-1,3,8-menthatriene	t	0.1	t	t	-
1118	cis-p-menth-2-en-1-ol	t	t	t	t	0.1
1122	α -campholenal	t	t	t	t	0.5
1132	cis-limonene oxide	t	0.2	t	t	-
1135	trans-pinocarveol	t	t	t	t	0.8
1137	trans-verbenol	0.2	0.1	t	0.3	0.2
1141	camphor	t	t	t	0.3	1.2
1145	camphene hydrate	-	-	-	-	0.1
1165	borneol	t	0.1	t	0.5	-
1166	p-mentha-1,5-dien-8-ol	t	t	t	t	-
1172	cis-pinocamphone	t	t	t	t	0.2
1174	terpinen-4-ol	0.2	t	t	0.3	0.1
1178	naphthalene	t	t	t	0.3	0.1
1179	p-cymen-8-ol	t	t	t	t	0.1
1186	α -terpineol	0.4	0.3	t	1.1	t
1204	verbenone	-	-	-	-	0.2
1215	trans-carveol	-	-	-	-	0.2
1218	endo-fenchyl acetate	-	-	-	-	0.1
1249	piperitone	-	-	-	-	0.1
1274	pregeijerene B	1.2	0.6	t	0.5	-
1284	bornyl acetate	0.4	0.2	t	0.7	0.4
1291	(2E,4Z)-decadienal	-	-	-	-	0.1
1319	(2E,4E)-decadienal	-	-	-	-	2.4
1387	β -bourbonene	t	t	t	t	0.1

KI	Compound	Abha, S. A.	Addis Alem	Kijabe, Kenya	Thika, Kenya	<i>excelsa</i> Turkey
1389	β -elemene	t	t	t	t	-
1390	7-epi-sesquithujene	-	-	-	-	0.1
1410	α -cedrene	-	-	-	-	0.8
1413	β -funebreene	-	-	-	-	0.7
1417	(E)-caryophyllene	3.0	0.8	0.3	1.0	-
1419	β -cedrene	-	-	-	-	0.5
1429	cis-thujopsene	-	-	-	-	0.3
1451	trans-muurolo-3,5-diene	-	-	-	-	0.1
1452	α-humulene	3.8	1.1	0.5	1.5	0.1
1454	(E)- β -farnesene	-	-	-	-	0.2
1469	β -acoradiene	-	-	-	-	0.2
1475	trans-cadina-1(6),4-diene	-	-	-	-	0.2
1480	germacrene D	2.2	0.5	0.2	0.7	0.6
1493	trans-muurolo-4(14),5-diene	-	-	-	-	0.2
1493	epi-cubebol	-	-	-	-	0.3
1496	valencene	-	-	-	-	0.3
1500	β -himachalene	-	-	-	-	0.1
1504	cuparene	-	-	-	-	0.1
1506	(Z)- α -bisabolene	-	-	-	-	0.1
1512	α -alaskene	-	-	-	-	0.2
1513	γ -cadinene	t	t	t	t	-
1514	cubebol	-	-	-	-	0.4
1521	trans-calamenene	-	-	-	-	0.2
1522	δ -cadinene	0.3	t	t	t	0.3
1532	γ -cuparene	-	-	-	-	0.2
1548	elemol	-	-	-	-	-
1574	germacrene D-4-ol	0.2	0.1	t	t	-
1589	allo-cedrol	-	-	-	-	1.9
1600	cedrol	0.4	-	t	-	25.4
1608	humulene epoxide II	0.6	0.3	0.3	0.7	t
1608	-oploponone	-	-	-	-	t
1627	1-epi-cubenol	-	-	-	-	0.5
1630	γ -eudesmol	0.8	0.6	0.3	1.5	-
1632	β -acorenol	-	-	-	-	0.1
1638	epi- α -cadinol	t	t	t	t	t
1640	epi- α -muurolol	t	t	t	t	t
1645	cubenol	-	-	-	-	0.1
1649	β -eudesmol	1.1	0.7	0.5	1.5	-
1652	α -eudesmol	1.6	0.9	0.7	2.2	-
1653	α -cadinol	-	-	-	-	t
1661	sesquiterpene, <u>85,57,41,238</u>	-	-	-	-	1.0
1668	β -atlantone	-	-	-	-	0.6
1670	bulnesol	0.5	0.3	t	0.6	-
1685	germacra-4(15),5,10-trien-1-al	t	0.1	t	0.4	-
1713	cedroxyde	-	-	-	-	0.1
1792	8- α -acetoxylemol	0.9	1.0	0.6	2.3	-
1958	iso-pimara-8(14),15-diene	t	0.2	t	0.3	-

KI	Compound	Abha, S. A.	Addis Alem	Kijabe, Kenya	Thika, Kenya	<i>excelsa</i> Turkey
1987	manoyl oxide	0.3	3.3	1.1	0.7	0.1
2055	abietatriene	0.2	0.2	0.5	1.4	t
2087	abietadiene	0.6	1.9	4.0	11.8	-
2105	iso-abienol	t	0.8	5.2	1.9	-
2153	abieta-8(14),13(15)-diene	t	t	0.1	0.3	-
2181	sandaracopimarinal	0.2	0.1	0.2	0.8	-
2269	sandaracopimarinol	t	0.1	0.2	0.6	-
2282	sempervirol	0.2	0.2	0.3	0.9	-
2298	4-epi-abietal	1.8	0.8	0.6	1.4	0.2
2314	trans-totarol	1.3	2.4	3.7	11.0	-
2331	trans-ferruginol	0.4	0.5	1.0	2.6	-
2401	abietol	0.2	0.1	t	0.2	-

Taxonomy of the *Dalea phleoides* (Fabaceae) complex

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ABSTRACT

Dalea phleoides is largely confined to eastern Texas, having first been proposed by Torrey & Gray in 1838 from collections gathered by Leavenworth; Shinnars (1949) separated from this *D. drummondiana*, the latter reduced to varietal status of *D. phleoides* by Barneby (1977) as *D. p. var. microphylla*. I follow the treatment of Shinnars, adding a new specific name to the complex, ***Dalea carrizoana*** B.L. Turner, **sp. nov.**, this, so far as known, largely confined to southern Texas. A photograph of the holotype is presented, along with maps showing distributions of the taxa concerned. Published on-line www.phytologia.org *Phytologia* 95(4): 274-278 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Fabaceae, *Dalea*, *D. drummondiana*, *D. phleoides*, *D. carrizoana*, Texas

Barneby (1977) presented a thorough overview of *D. phleoides* (Torr. & Gray) Shinnars and *D. drummondiana* Shinnars, treating these as but varieties under his broad concept of the former, although admitting that the two taxa “are found to have substantially different but at the same time widely overlapping ranges of dispersal and where they overlap a common habitat.” Barneby used the following key to distinguish the two taxa:

1. Primary cauline leaves with 6-10 (12) pairs of leaflets up to 5-14 mm long;
axis of spike and exterior of calyx glabrous or almost so.....**var. phleoides**
1. Primary cauline leaves with 12-20 (24) pairs of leaflets up to 2-7 mm long;
axis of spike and calyx-tube, at least at base, often throughout,
having pilosulous, spreading-incurved, hairs.....**var. microphylla**

In the present treatment, I recognize three species within the populational complex concerned, as follows:

1. Leaves with leaflets mostly 6-14 mm long; calyces mostly 3.0-3.5 mm long;
far eastern Texas.....**D. phleoides**
1. Leaves with leaflets mostly 2.5-6.0 mm long; calyces 2.5-3.0 mm long.....**(2)**
2. Leaves (mostly), and axis of inflorescence pubescent; calyx tubes
pubescent; eastern Texas.....**D. drummondiana**
2. Leaves, and axis of inflorescence glabrous; calyx tubes
glabrous; southern Texas.....**D. carrizoana**

DALEA CARRIZOANA B.L. Turner, **sp. nov.** **Fig. 1**

Perennial herbs, 30-60 cm high, branched basally from deep ligneous taproots. **Stems** glabrous, glandular-punctate. **Leaves** (mid-stem), 2-5 cm long, glabrous throughout, odd-pinnate, with 5-9 pairs of lateral leaflets, 3-7 mm long, 0.5-1.0 mm wide, upper surfaces eglandular, the terminal leaflets 1-2 mm longer than the laterals. **Inflorescences** (the petals excluded), 6-9 mm wide, 3-6 cm long, the floral axis glabrous; peduncles glabrous, 10-18 cm long. **Calyx** glabrous, 2.5-3.0 mm long, glandular-pustulate throughout; tubes 2.0-2.5 mm long, the lobes ca 0.6 mm long. **Petals** white, the banner ca 5 mm long, claw ca 2.5 mm long, the blade cordate. **Pods** ca 2 mm high, 2 mm wide, laterally pilosulous to glabrate.

TYPE: U.S.A. TEXAS. DIMMIT CO., “Deep sandy soil on Carrizo sand outcrop near Carrizo Springs.” 2 May 1954, *B.C. Tharp & M.C. Johnston 3515* (Holotype: TEX).

ADDITIONAL SPECIMENS EXAMINED: TEXAS. CALDWELL CO. (?): w/o locality, Spring-summer, 1931, *MacBride s.n.* (TEX). **DE WITT CO.**: western part of county, 20 Jul 1941, *Riedel s.n.* (TEX). **DIMMIT CO.**: ca 3.5 mi W of Carrizo Springs, 29 Jun 1899, *Bray (?) s.n.* (TEX); 14 mi NW of Carrizo Springs along route 277, 8 Jul 1958, *Correll & Johnston 19479* (LL). **KARNES CO.**: ca Ecletto Creek, hwy 627, 25 Jul 1952, *Johnson 998* (TEX); 2.5 mi NE Panna Maria, 6 Jun 1953, *Johnson 1259* (TEX). **WILSON CO.**: “Kicaster School.” 24 Jun 1935, *Cory 15076* (TEX).

Dalea carrizoana is readily distinguished from *D. phleoides* by its smaller leaves and narrower flowering spikes (6-9 mm wide vs 10-11 mm wide); it is also quite different from the more closely allopatric, *D. drummondiana*, in having glabrous calyx tubes and a glabrous floral axis, as noted in the above key.

Wemple (by annotation, TEX) included specimens of the present novelty within his concept of *D. phleoides*, as did Turner (1959) in his treatment of *The Legumes of Texas*. Barneby (1977) did not cite or annotate the sheets concerned, presumably not having examined them. The Caldwell county specimen is queried since it is likely that the collection was made elsewhere by the collector concerned, perhaps in more southern outcrops of the Carrizo sands.

The novelty is named for the city of Carrizo Springs, near from which was obtained type material. While the type of the species was obtained from the Carrizo sands near that municipality, it seems not confined to the latter substrate (as does *Hymenopappus carrizoanus*, Turner et al., 2003), hence my reluctance to base the name on that well known geological outcrop. Distribution of the taxon is shown in Fig. 2.

It should be noted that the species is apparently quite rare; attempts to re-collect the taxon in Dimmit Co. in the late spring of 2013 proved unsuccessful, in spite of several hours of roadside searching.

DALEA DRUMMONDIANA Shinnery, Field & Lab. 17: 83. 1949.

Dalea phleoides var. *microphylla* (Torr. & Gray) Barneby

Kuhnistera microphylla (Torr. & Gray) A. Heller

Petalostemon microphyllus (Torr. & Gray) A. Heller

Petalostemon phleoides var. *microphyllum* Torr. & Gray

This taxon is relatively common throughout most of east Texas (Fig.3), and is largely sympatric with its closest relative, *D. phleoides* (Fig. 2). The two species, so far as known, have not been collected growing together, and both are relatively well marked, hence their treatment as species, much as Wemple (1970) accorded the taxa. Barneby (1977), however, treated the two taxa as but varietally distinct.

Specimens of *D. drummondii* usually have leaflets to some degree pubescent; however, a single collection of the latter from Brown Co., Texas, having leaflets glabrous throughout was incorrectly annotated by Barneby (TEX) as *D. leporina*, a species of Trans-Pecos Texas, which it superficially resembles.

DALEA PHLEOIDES (Torr. & Gray) Shinnery, Field & Lab. 17: 83. 1949.

Dalea glandulosa (Coulter & Fisher) Shinnery, not *Dalea glandulosa* (Blanco) Merrill

Kuhnistera phleoides (Torr. & Gray) O. Kze.

Petalostemon glandulosus Coulter & Fisher

My treatment of this taxon is about the same as that of Barneby (1977), with the exception of *D. drummondiana*. Barneby failed to recognize *D. carrizoana*, not having examined, so far as known, any of the several sheets cited herein. Wemple (1970) identified several of the *D. carrizoana* sheets at TEX as *D. phleoides*, presumably because of the glabrous floral axis and calyces, such as occurs in the latter. Regardless, I find *D. carrizoana* quite distinct, possessing characters of both *D. phleoides* and *D. drummondiana*, having fewer leaflets as in the former, but the habit and smaller leaflets of the latter.

The type of *D. phleoides* was reportedly collected in the state of Arkansas by Leavenworth; however, Barneby (1977) noted that type material was most likely obtained by Leavenworth in his travels through eastern Texas during the years 1834 or 1837.

ACKNOWLEDGEMENTS

The dot maps are based upon specimens on file at LL, TEX, and those cited by Barneby (1977). My long time companion, Jana Kos, accompanied me in the field, and edited the paper providing helpful suggestions.

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Fig. 1. *Dalea carrizoana* (holotype).

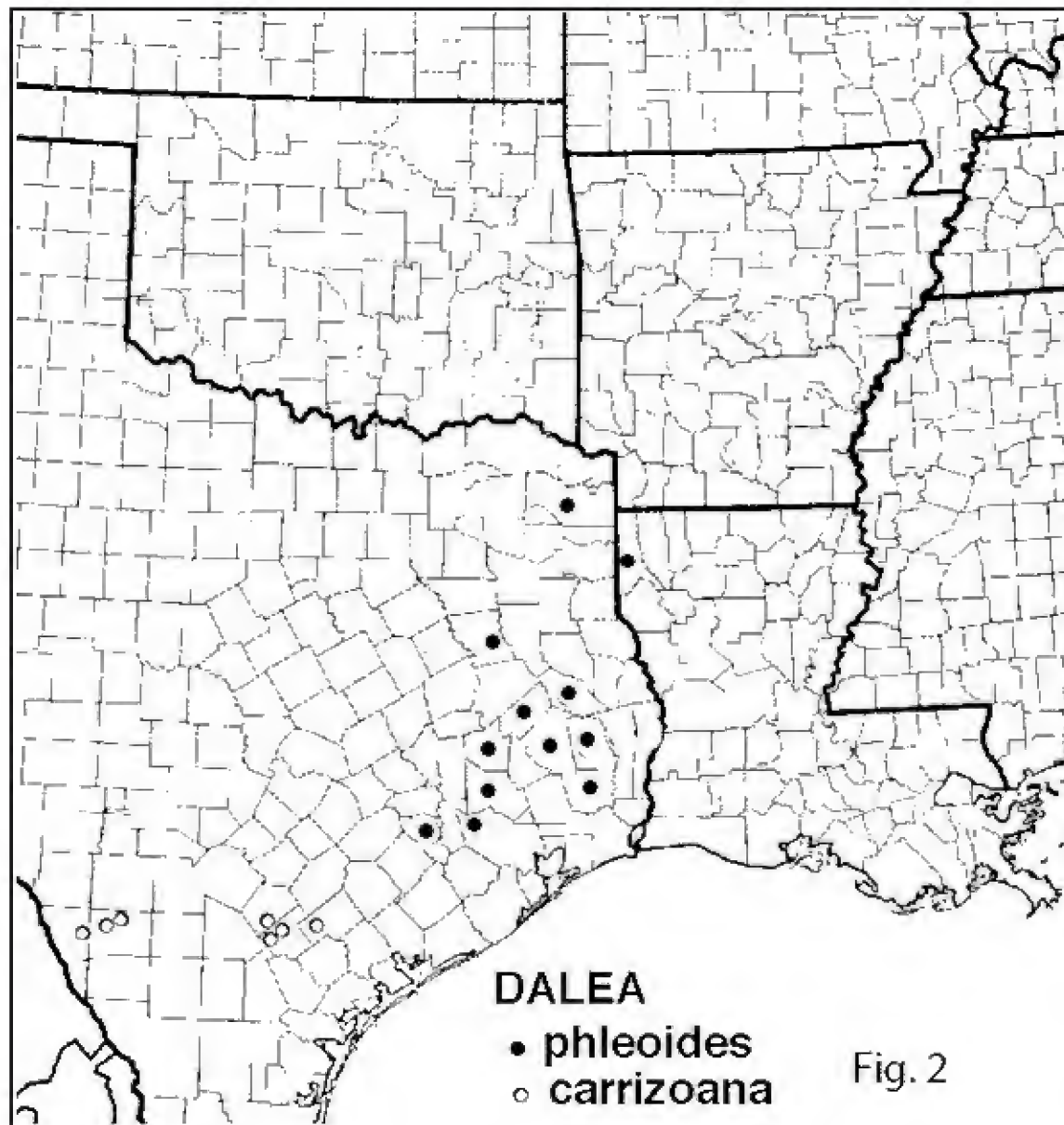


Fig. 2. Distribution of *Dalea carrizoana* and *D. phleoides*.

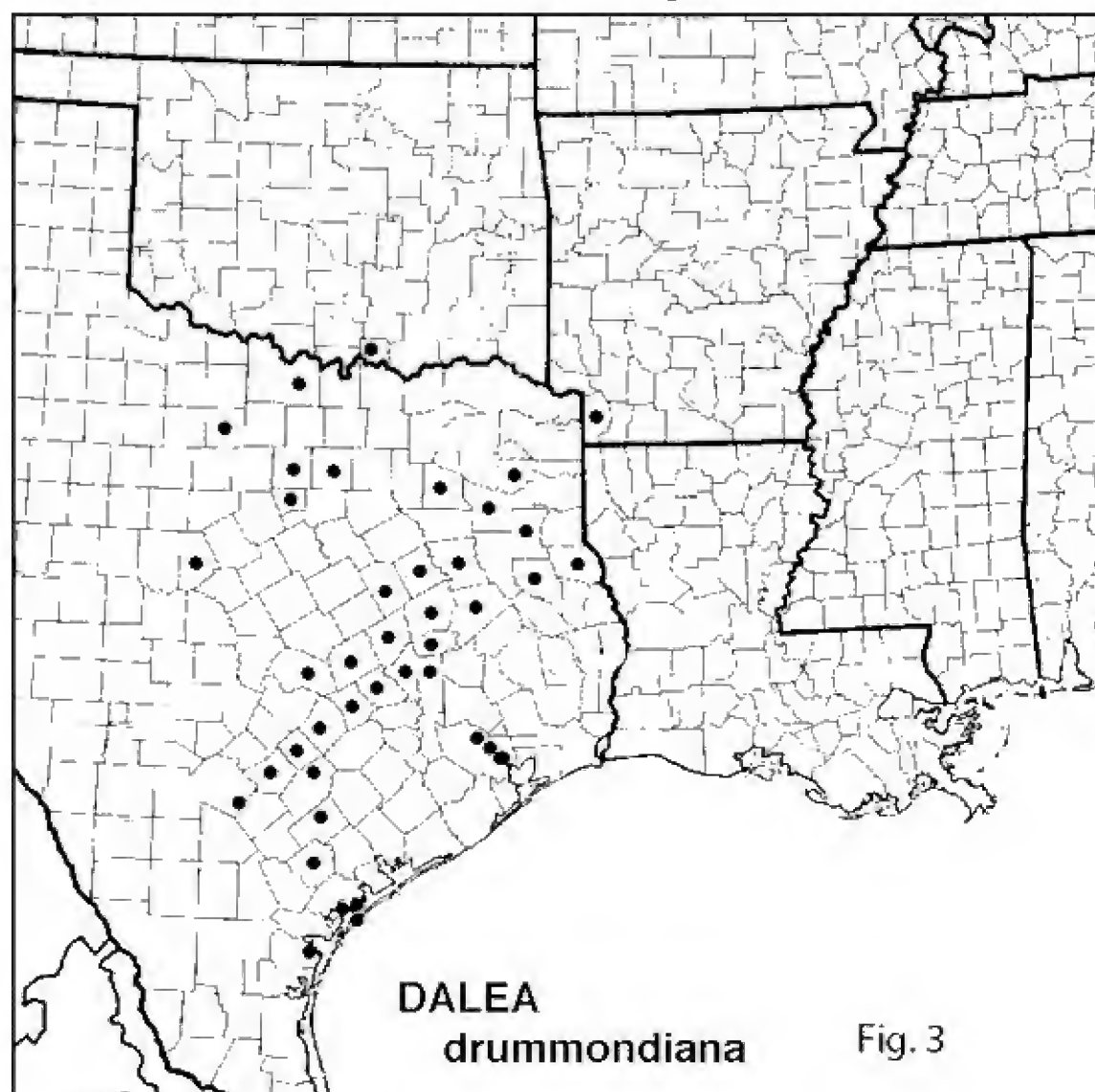


Fig. 3. Distribution of *Dalea drummondiana*.

Geographic variation in the volatile leaf oils of *Juniperus excelsa* M.-Bieb.**Robert P. Adams**

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ABSTRACT

Comparisons of the volatile leaf oil components for four populations of *J. excelsa* are reported. The major components are cedrol (25.4 - 29.3%), α -pinene (21.6 to 41.8%), limonene (0.5 - 13.2%), β -phellandrene (0.4 - 9.2%) and (2E,4E)-decadienal (2.3 - 3.9%). The trees from Greece and Bulgaria were higher in limonene (13.2, 11.3%) and β -phellandrene (13.2, 11.3%) than the samples from Turkey and Cyprus. In contrast, trees from Turkey and Cyprus were higher in α -pinene (41.7, 32.6%) than trees in Greece and Bulgaria (21.6, 24.3%). Fifteen compounds, normally found only in heartwood, were present in the leaf oil, with cedrol being the major component. Cedrol ranged, rather continuously, from 11.3 to 35.8%, with no chemical polymorphisms detected among 12 trees from 3 regions. Published on-line: www.phytologia.org *Phytologia* 95(4): 279-285 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: *Juniperus excelsa*, geographic variation, leaf oils, terpenes, cedrol, α -pinene, limonene.

Juniperus excelsa M.-Bieb. is a wide-ranging species (Fig. 1) from Greece to Turkey and perhaps far east as Azerbaijan. Farjon (2005, 2010) treated *J. polycarpos*, *J. p.* var. *seravschanica* and *J. p.* var. *turcomanica* as *J. excelsa* subsp. *polycarpos*. However, Adams and Schwarzbach (2012) and Adams (2013), utilizing DNA sequence data, recognized *J. excelsa* as well as *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica*. Adams and Hojjati (2012), using sequences from 4 gene regions, failed to find *J. excelsa* in Iran, but did find *J. polycarpos*, *J. p.* var. *turcomanica* and *J. seravschanica* in Iran. Putative *J. excelsa* from Qushchi, in extreme northwest Iran, had 0 or only 1 SNP difference compared with *J. polycarpos* var. *polycarpos* from Armenia (Adams and Hojjati, 2012).

The early papers on volatile leaf oils have been reported upon, and literature reviewed by Adams (1990a). More recently, Ucar and Balaban (2002) examined the volatile oil from sapwood (outside white wood next to the interior heartwood) from *J. excelsa* and reported 22.5% widdrol and 9% cedrol. The volatile oil from berries (seed cones) of *J. excelsa* is generally similar to leaf oil and Unlu et al. (2008) found berry oil from Turkey to contain 55.5% α -pinene, 7.75% cedrol, 3.55% sabinene along with 51 other compounds.

The purpose of this paper is to present an updated analyses of the oils and geographic variation of *J. excelsa*.

MATERIALS AND METHODS

Plant material - *J. excelsa*: Eskisehir, Turkey, Adams 13193 (9433-9435), Bulgaria, Adams 14056 (13720-13724), Alex Tashev, 2012-1-JE -5-JE, 42° 01' 22.0" N; 24° 28' 03.1" E, 356 m, Central Rhodopes, above the town of Kritchim, Reserve "Izgorialoto Gune", Lemos, Greece, Adams 6031 (5983-5985, 5987), Cyprus, Adams 13487, Bouchra Douaihy ns., bulk 5 trees. *J. procera*: Adams 6184-6188, near Addis Alem, Ethiopia, 40 km west of Addis Ababa on road to Guder, 2400 m, ca. 9° 02' N, 38° 23' E. Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

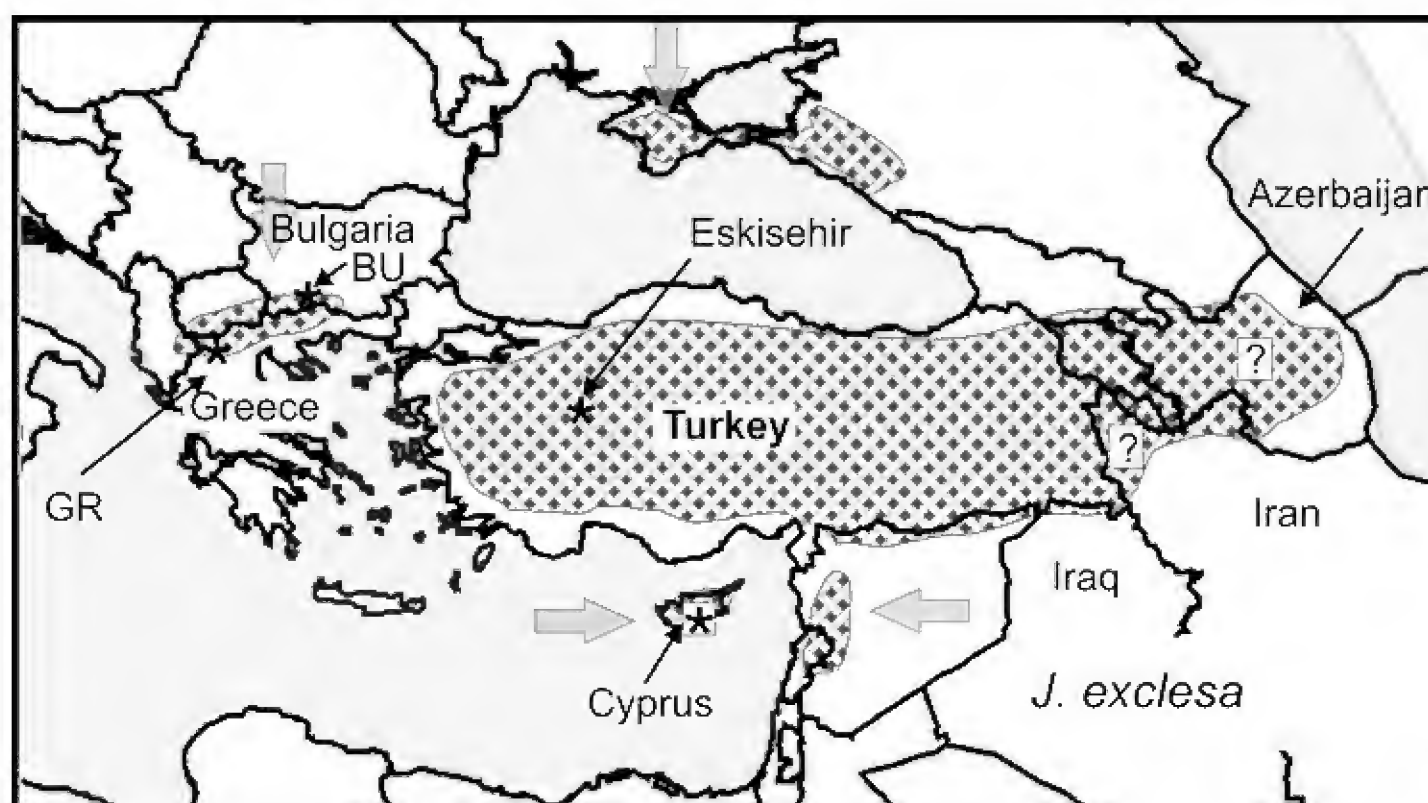


Figure 1. Distribution of *J. excelsa* from Adams (2011) with populations sampled in this study. Question marks (?) indicate questionable occurrences of *J. excelsa* in Azerbaijan and Iran.

Fresh or air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

RESULTS AND DISCUSSION

Comparisons of the leaf components for the four populations are given in Table 1. The major components of the oils are cedrol (25.4 - 29.3%), α -pinene (21.6 to 41.8%), limonene (0.5 - 13.2%), β -phellandrene (0.4 - 9.2%) and (2E,4E)-decadienal (2.3 - 3.9%%). The trees from Greece and Bulgaria are higher in limonene (13.2, 11.3%) and β -phellandrene (13.2, 11.3%) than the samples from Turkey and Cyprus (Table 1). In contrast, trees from Turkey and Cyprus were higher in α -pinene (41.7, 32.6%) than trees in Greece and Bulgaria (21.6, 24.3%).

In general, most *Juniperus* species produce two kinds of essential oils: leaf oils and heartwood oils and these oils have few components in common (Adams, 1991). *Juniperus excelsa*, along with *J. foetidissima*, *J. polycarpos* *J. p.* var. *turcomanica* and *J. seravschanica* have leaf oils that contain significant amounts of the heartwood oil components (cf. α -cedrene β -cedrene, thujopsene, cuparene, cedrol, widdrol, etc., see Adams, 2011). For example, Adams (1990b) reported 4.4, 0.2, trace and 8.3% cedrol in the leaf oils from four trees of *J. foetidissima* from Greece. Whereas, Tunalier et al. (2004) reported 13.0 and 12.2% of cedrol and widdrol in the stem heartwood of *J. foetidissima* from Turkey. Ucar and Balaban (2002) analyzed the sapwood (white wood) of *J. excelsa*, Turkey, and reported the oil to contain 22.5% widdrol and 9.0% cedrol (these components are difficult to separate on non-polar columns and the mass spectra are nearly identical, so identification is often problematic). In the present case, *J. excelsa* leaf oils contain 15 compounds normally restricted to heartwood (boldface, Table 1). The trend from Greece-Bulgaria to Turkey-Cyprus is not seen in the heartwood components, as they are mostly uniform across the samples in this study.

When *Juniperus* species do contain heartwood components in the leaf oils, it is common to find chemical polymorphisms in cedrol between trees (see discussion of *J. foetidissima* above with 4.4, 0.2, trace and 8.3% of cedrol in leaf oils). However, in this study, the concentration of cedrol in the leaf oils is fairly continuous from 11.3% to 35.8% among the 12 individual trees examined (Table 2). The trees in the Bulgaria population were very uniform, ranging from 21.0 to 28.2% cedrol (Table 2).

The leaf oil of *J. procera* is included in table 1 for a comparison. It differs from *J. excelsa* oil in many components including δ -3-carene, terpinolene, linalool, (E)-caryophyllene, α -humulene, γ -eudesmol, 8- γ -acetoxyelemol, and the presence of 10 diterpenes not found in *J. excelsa*. In addition, its leaf oil is, as found in most junipers, without the heartwood components (Table 1).

ACKNOWLEDGEMENTS

Thanks to Billie Turner for review. Thanks to Tonya Yanke for lab assistance. This research was supported in part with funds from Baylor University.

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Table 1. Comparison of leaf essential oils from populations of *J. excelsa*. Bulgaria: Adams 14056 (13720-13724); Lemos, Greece: Adams 6031 (5983-5985, 5987), Eskisehir, Turkey; Adams 13193 (9433-9435) and Cyprus, Adams 13487 (bulk, 5 trees). Components that are typical of heartwood oil are highlighted in boldface. The oil of *J. procera* is from Addis Alem, Ethiopia, Adams 6184-6188.

KI	Compound	Bulgaria	Greece	Eskisehir	Cyprus	<i>procera</i>
921	tricyclene	t	0.1	0.2	0.1	t
932	α -pinene	24.3	21.6	41.7	41.8	32.6
945	α -fenchene	0.1	0.1	0.3	t	1.1
946	camphene	0.2	0.3	0.2	0.1	0.2
953	thuja-2,4(10)-diene	t	t	0.1	t	t
961	verbenene	t	t	t	t	t
969	sabinene	t	0.2	0.1	0.1	t
974	β -pinene	0.5	0.5	0.7	0.4	3.1
988	myrcene	1.4	1.2	1.2	0.5	3.1
1002	α -phellandrene	t	t	0.1	t	t
1008	δ -3-carene	0.8	1.7	5.3	t	28.6
1014	α -terpinene	t	t	0.1	t	t
1020	p-cymene	0.5	0.4	0.6	0.1	0.2
1024	limonene	11.3	13.2	1.2	0.5	0.5
1025	β -phellandrene	7.5	9.2	0.9	0.4	1.4
1044	(E)- β -ocimene	t	t	t	t	t
1054	γ -terpinene	0.5	0.4	0.5	0.3	t
1065	cis-sabinene hydrate	t	t	t	t	-
1086	terpinolene	0.8	0.7	1.1	0.3	3.2
1095	linalool	-	-	-	-	0.6
1097	trans-sabinene hydrate	t	t	t	t	-
1112	3-me-3-butenyl-methyl butanoate	0.4	0.1	t	t	-
1112	endo-fenchol	0.2	0.2	t	t	-
1118	cis-p-menth-2-en-1-ol	-	-	0.1	t	t
1122	α -campholenal	0.2	0.1	0.5	0.1	t
1132	cis-limonene oxide	-	-	-	-	0.2
1135	trans-pinocarveol	0.4	0.2	0.8	0.1	t
1137	trans-verbenol	-	-	0.2	-	0.1
1141	camphor	0.7	0.5	1.2	0.2	t
1145	camphene hydrate	t	t	0.1	t	-
1165	borneol	-	-	-	-	0.1
1166	p-mentha-1,5-dien-8-ol	t	t	-	t	t
1172	cis-pinocamphone	t	t	0.2	t	t
1174	terpinen-4-ol	t	0.2	0.1	0.1	0.2
1178	naphthalene	t	t	0.1	t	t
1179	p-cymen-8-ol	t	t	0.1	t	t
1186	α -terpineol	t	t	t	t	0.4
1204	verbenone	t	t	0.2	t	-
1215	trans-carveol	0.2	0.1	0.2	t	-
1218	endo-fenchyl acetate	t	0.2	0.1	t	-
1249	piperitone	t	t	0.1	t	-

KI	Compound	Bulgaria	Greece	Eskisehir	Cyprus	<i>procera</i>
1260	3-me-3-butenol hexanoate	0.2	0.1	-	-	-
1274	pregeijerene B	-	-	-	-	0.6
1284	bornyl acetate	t	0.6	0.4	0.1	0.2
1292	(2E,4Z)-decadienal	t	0.3	0.1	t	-
1319	(2E,4E)-decadienal	3.9	3.6	2.4	2.3	0
1387	β -bourbonene	t	0.1	0.1	t	t
1389	β -elemene	-	-	-	-	t
1390	7-epi-sesquithujene	0.2	0.1	0.1	0.3	-
1410	α-cedrene	1.0	1.1	0.8	1.7	-
1413	β-funebreene	1.0	0.9	0.7	1.8	-
1417	(E)-caryophyllene	-	-	-	-	0.8
1419	β-cedrene	1.1	1.0	0.5	1.0	-
1429	cis-thujopsene	0.3	0.4	0.3	0.8	-
1451	trans-muurolo-3,5-diene	0.2	0.1	0.1	0.7	-
1452	α -humulene	0.1	0.2	0.1	0.2	3.8
1454	(E)- β -farnesene	0.1	0.2	0.2	0.3	-
1469	β-acoradiene	0.1	0.1	0.2	0.4	-
1475	trans-cadina-1(6),4-diene	0.4	0.3	0.2	0.7	-
1480	germacrene D	0.8	0.8	0.6	1.2	2.2
1493	trans-muurolo-4(14),5-diene	0.6	0.4	0.2	1.5	-
1493	epi-cubebol	-	-	0.3	-	-
1496	valencene	0.6	0.5	0.3	t	-
1500	β-himachalene	-	t	0.1	-	-
1504	cuparene	-	t	0.1	t	-
1506	(Z)-α-bisabolene	-	-	0.1	t	-
1512	α-alaskene	0.4	0.3	0.2	0.2	-
1513	γ -cadinene	-	-	-	-	t
1514	cubebol	0.8	0.7	0.4	1.2	-
1521	trans-calamenene	0.5	0.2	0.2	0.5	-
1522	δ -cadinene	0.5	0.3	0.3	0.6	0.3
1532	γ-cuparene	0.3	0.2	0.2	0.5	-
1574	germacrene D-4-ol	-	-	-	-	0.1
1589	allo-cedrol	1.7	2.0	1.9	2.4	-
1600	cedrol	25.5	29.3	25.4	27.5	-
1608	humulene epoxide II	t	t	t	t	0.3
1608	β -oplophenone	t	t	t	t	-
1627	1-epi-cubenol	0.8	0.7	0.5	0.7	-
1630	γ -eudesmol	-	-	-	-	0.6
1632	β-acoreanol	t	0.1	0.1	0.2	-
1638	epi- α -cadinol	t	t	t	t	-
1640	epi- α -muurolol	t	t	t	t	-
1645	cubenol	t	t	0.1	t	-
1649	β -eudesmol	-	-	-	-	1.1
1652	α -eudesmol	-	-	-	-	1.6
1653	α -cadinol	t	t	t	t	-
1661	sesquiterpene <u>85,57,41,238</u>	1.1	0.9	1.0	1.2	-
1668	β-atlantone	0.7	0.5	0.6	0.7	-
1670	bulnesol	-	-	-	-	0.3

KI	Compound	Bulgaria	Greece	Eskisehir	Cyprus	<i>procera</i>
1685	germacra-4(15),5,10-trien-1-al	-	-	-	-	0.1
1713	cedroxyde	-	-	0.1	t	-
1792	8- α -acetoxyelemol	-	-	-	-	0.9
1958	iso-pimara-8(14),15-diene	-	-	-	-	t
1987	manoyl oxide	t	0.2	0.1	t	3.3
2055	abietatriene	t	0.4	t	t	0.2
2087	abietadiene	-	-	-	-	1.9
2105	iso-abienol	-	-	-	-	0.8
2153	abieta-8(14),13(15)-diene	-	-	-	-	t
2181	sandaracopimarinal	-	-	-	-	0.1
2269	sandaracopimarinol	-	-	-	-	t
2282	sempervirol	-	-	-	-	0.2
2298	4-epi-abietal	0.2	0.2	0.2	t	0.8
2314	trans-totarol	-	-	-	-	2.4
2331	trans-ferruginol	-	-	-	-	0.5
2401	abietol	-	-	-	-	0.1

Table 2. Variation in cedrol (% total oil) among individual trees in populations of *J. excelsa*. Note that the Cyprus value for cedrol is from bulked leaves from 5 trees.

Bulgaria	22.1	24.4	26.0	28.2	24.4
Greece	23.0	18.8	28.0	35.8	
Eskisehir	27.6	11.3	22.1		
Cyprus	27.5				

Heliomeris multiflora* var. *macrocephala* (Asteraceae: Heliantheae), A new record from the U.S.A.*Billie L. Turner**

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ABSTRACT

A new record of *H. multiflora* var. *macrocephala* from Cochise Co. AZ is reported.
Published on-line www.phytologia.org *Phytologia* 95(4): 286-287 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: *Heliomeris multiflora* var. *macrocephala*, new record, Arizona.

In my recent account of the Mexican species of *Heliomeris* (Turner, 2012a), I called attention to a single collection of *H. m.* var. *macrocephala* Heiser from the U.S.A. (Map 1), the full record of which follows:

U.S.A.: ARIZONA. COCHISE CO.: “along the Bear Canyon trail, T23S R20E Sec. 29 NE1/4;” shaded slope in canyon bottom woodland with *Pseudotsuga menziesii*, 7000 ft, 29 Sep 1991, *Bowers & McLaughlin* 3606 (ARIZ).

The specimen appears to be a high elevational relict of what once was a more widespread, subalpine populational system of *H. multiflora*, its southern counterpart having been collected but sparingly in spruce-fir forests at somewhat higher elevations on Sierra Mohinora, Chihuahua, as noted by Turner (2012a).

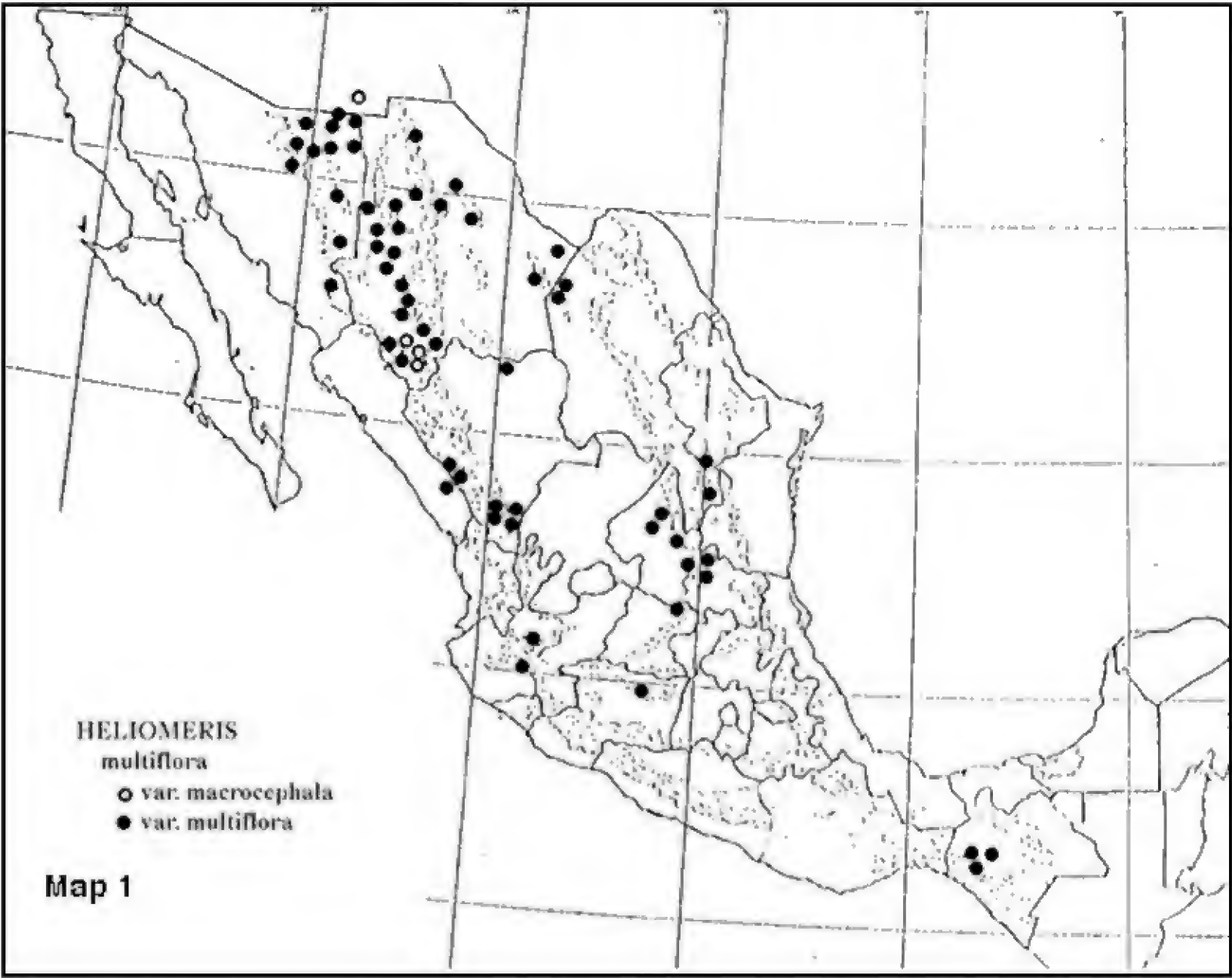
Interestingly, the present high elevation relict population is similar to that called to the fore in the genus *Senecio* by Turner (2012b) in which *S. multidentatus*, previously known only from sporadic, high elevational sites throughout northern Mexico, was reported to have a single collection from the U.S.A. [Cochise Co., Chiricahua Wilderness Area, Chiricahua Mts., “Snowshed Trail; coniferous forest,” 8750 ft, 18 Sep 1976, *Leithliter* 829 (ASU)]. Turner notes, “it is interesting to speculate that the ancestral populations of *S. multidentatus* that might have given rise to *S. huachucanus* are still represented in Arizona by relictual populations in the Chiricahua Mts. DNA should ultimately help resolve the problem.” The same might be said for the enigmatic *Heliomeris* var. *macrocephala*!

ACKNOWLEDGEMENTS

I am grateful to my colleague Guy Nesom and my field companion, Jana Kos, for editorial comments. The following herbaria loaned appropriate specimens: ARIZ, ASU, LL-TEX.

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Map 1. Distribution of *Heliomeris multiflora* var. *macrocephala*.

Leaf essential oils of *Juniperus* in central and southern Iran

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ABSTRACT

Leaf essential oils from *Juniperus* from southern Iran were analyzed and compared to oils of *J. excelsa*, *J. polycarpus*, *J. p. var. turcomanica* and *J. seravschanica*. The juniper oils from southern Iran were mainly in two groups: high cedrol (cf. *J. excelsa*, *J. polycarpus* and *J. seravschanica*) and low cedrol (cf. *J. p. var. turcomanica*). Complete analyses of the compositions are given.

Published on-line www.phytologia.org *Phytologia* 95(4): 288-295 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: *Juniperus polycarpus* var. *polycarpus*, *J. seravschanica*, *J. p. var. turcomanica*, *J. excelsa*, Cupressaceae, Iran, terpenes, leaf essential oil.

The distributions of *J. excelsa* M.-Bieb., *J. polycarpus* K. Koch and *J. seravschanica* Kom. in Iran and the surrounding region are not well understood (Adams, 2011). Figure 1 summarizes our current understanding of these taxa's distributions. Adams and Hojjati (2012) investigated 10 populations of *Juniperus* in Iran using nrDNA, petN-psbM, trnD-trnT and trnS-trnG sequences (3705 bp). They found the northern populations, BL, Bj, Sh, were in a clade with *J. p. var. turcomanica* and L, H, and Q in a clade with *J. polycarpus* (Fig. 2). However, the southern populations displayed a mosaic pattern. One of

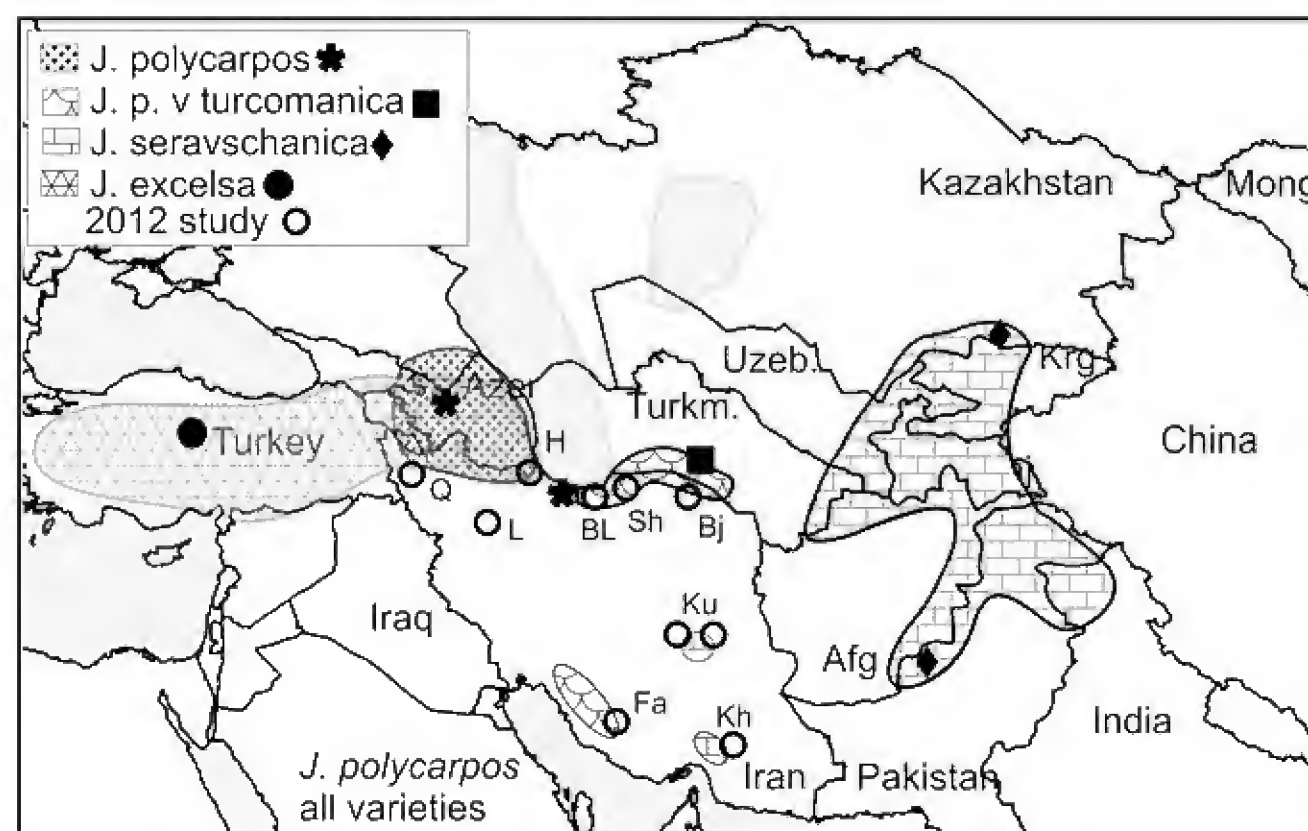


Figure 1. Distributions of *J. excelsa* (Greece not shown), *J. polycarpus* var. *polycarpus*, *J. p. var. seravschanica*, *J. p. var. turcomanica*. (adapted from Adams and Hojjati, 2012, symbols indicate the populations sampled for each taxon).

the samples from southern Kuhbanan population (Ku1) is loosely associated with the northern clade and a second sample (Ku2) is in a clade with Khabr (Kh1, Kh2) that is associated with *J. seravschanica*. Another perspective is shown in Figure 3, where the northern populations, L, H, Q, are clearly associated

with *J. polycarpus*. Other northern populations BL, Bj, Sh, are loosely linked to var. *turcomanica* (Fig. 3). The Fasa samples (F1, F2) differ by only one SNP from BL in northern Iran. The samples from Khabr (Kh1, Kh2) are linked to *J. seravschanica* by 9 SNP differences (Fig. 3) and a sample from Kuhbanan (Ku2) is linked to the Khabr samples by 6 SNPs differences (Fig. 3). Clearly the junipers from southern Iran contain elements of *J. p.* var. *turcomanica* and *J. seravschanica*.

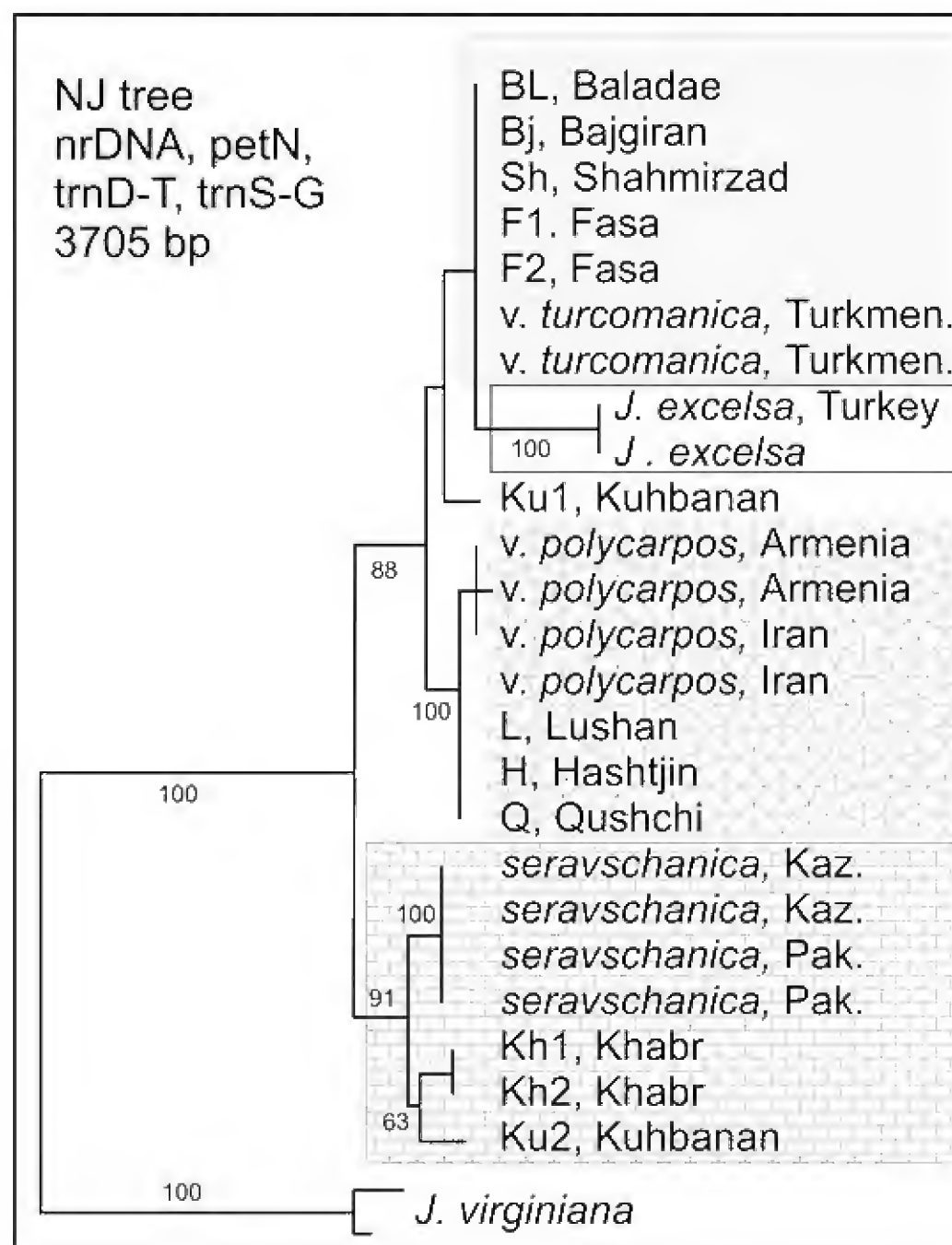


Figure 2. NJ tree of Iranian junipers (adapted from Adams and Hojjati, 2012).

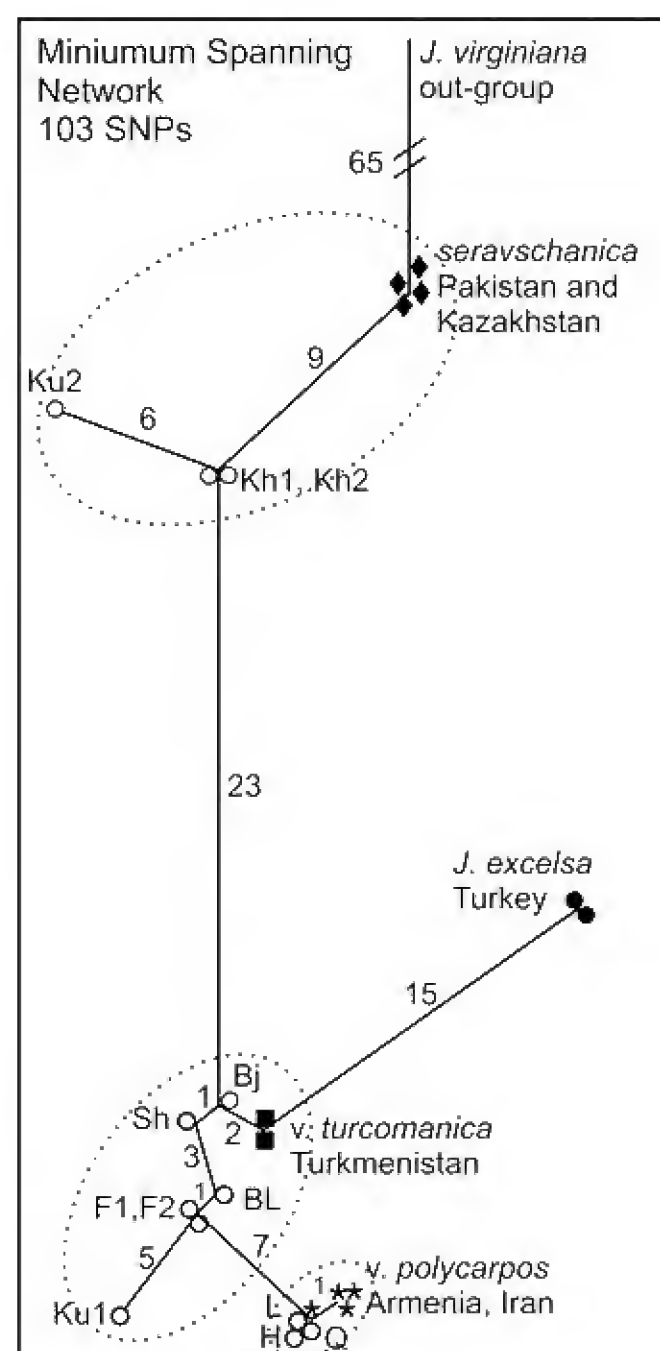


Figure 3. Minimum spanning network (adapted from Adams and Hojjati, 2012).

Adams and Shanjani (2011), using DNA sequence data, showed the juniper from the Elburz Mtns. to be typical *J. polycarpus* not *J. excelsa* as supposed. The composition of the leaf oils of *J. polycarpus* and *J. seravschanica* were previously reported by Adams (2001) and Adams et al. (2008). The leaf essential oils of *J. excelsa* have recently been reported (Adams et al. 2013).

The purpose of the present study is to investigate the leaf essential oils of the southern *Juniperus* of Iran.

MATERIALS AND METHODS

Plant materials (see Fig. 4):

Fasa (F1-F5), putative *J. polycarpus* var. *turcomanica*, 30 km past Fasa towards Neiriz, common on rocks. 29° 09' 51.1" N; 53° 44' 13.5" W, 1715 m, Oct. 2012, Prov. Fars, F. Hojjati #1 to #5 (lab acc. Adams 13754-13758);

Kuhbanan (Ku)(K1-K9), putative *J. seravschanica* and *J. polycarpus* var. *turcomanica*, Kuh-e Bajgen, 55 km from Kuhbanan, Dolatabad, common on rocks. 31° 27' 12.8" N; 55° 52' 28.8" W, 2333 m, Oct. 2012, Kerman Prov., *F. Hojjati* *1 to *9 (lab acc. Adams 13759-13767);

Khabr(KH)(B1-B5), putative *J. seravschanica*, Kuh-e Khabr. common on rocks. 28° 49' 06.7" N; 56° 21' 21.7" W, 2086 m, Oct. 2012, Prov. Kerman, *F. Hojjati* -1 to -5 (lab acc. Adams 12768-13772);

Rabor (R)(R1-R5), putative *J. polycarpus* var. *turcomanica*, Gusichai village, 23 km past Rabor, between Rabor and Darbehest. 28° 49' 06.7" N; 56° 21' 21.7" W, 2086 m, Oct. 2012, Prov. Fasa, *F. Hojjati* .1 to .5 (lab acc. Adams 13773-13777).



Figure 4. Populations sampled in the present study.

Authentic, typical taxonomically identifiable reference taxa, were included from *J. excelsa*, n of Eskisehir, Turkey, Adams 9433-9435; *J. polycarpus* var. *polycarpus*, Lake Sevan, Armenia, Adams 8761-8763, *J. p.* var. *turcomanica*, Kopet Mtns., ca 140 km wnw of Ashgabat, Turkmenistan, 38° 25.12' N, 56 58.80' E, 1535m, Adams 8757-8760; *J. seravschanica*, Quetta, Pakistan, Adams 8483-8485, Dzhabagly, Kazakhstan. Voucher specimens are deposited at Baylor University (BAYLU).

Chemical analysis

Fresh, air dried leaves or herbarium specimens (20-100 g) were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (ether trap removed) with nitrogen and the samples stored at -20°C until analyzed. The extracted leaves were oven dried (100°C, 48 h) for determination of oil yields.

The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software. Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using the formulation of Gower (1966) and Veldman (1967).

RESULTS AND DISCUSSION

The volatile leaf oils of the junipers of southern Iran were of three kinds and illustrated (Table 1) by samples Kuh6 (high cedrol), Kuh9 (low cedrol) and Kbr5 (low cedrol). All the oils were high in α -pinene (48.7 - 62.5%). Kuh6 (23.6% cedrol) was more like *J. excelsa*, (25.4), *J. polycarpus* (30.3) and *J. seravschanica* (13.8, 22.7) in having high amount of cedrol (Table 1). In contrast, Kuh9 (0.0 cedrol) and Kbr5 (0.1 cedrol) were like *J. p.* var. *turcomanica* (0.2% cedrol). However, several terpenes in southern junipers were in higher concentrations than found in *J. excelsa*, *J. polycarpus* or *J. seravschanica*: limonene, β -phellandrene, trans-verbenol, α -eudesmol, and β -eudesmol (Table 1).

To further examine the patterns, the similarity matrix was factored and yielded eigenroots accounting for 29.3, 11.1 and 7.1% of the variation among the 24 samples plus the 5 exemplar taxa. The eigenroots appeared asymptote after the third root. The large amount of variance extracted by the first eigenroot indicates that the major trend was the separation of 2 groups (high and low cedrol oils) on the first eigenvector (Fig. 5). The second eigenroot (11%) mostly separates the exemplar taxa from the 24 southern Iran samples (Fig. 5). The third eigenroot (7%) separates a sub-group of low cedrol trees from the bulk of the low cedrol trees (Fig. 5). A close examination of individuals reveals that both high and low cedrol oils are found in each of the four populations sampled.

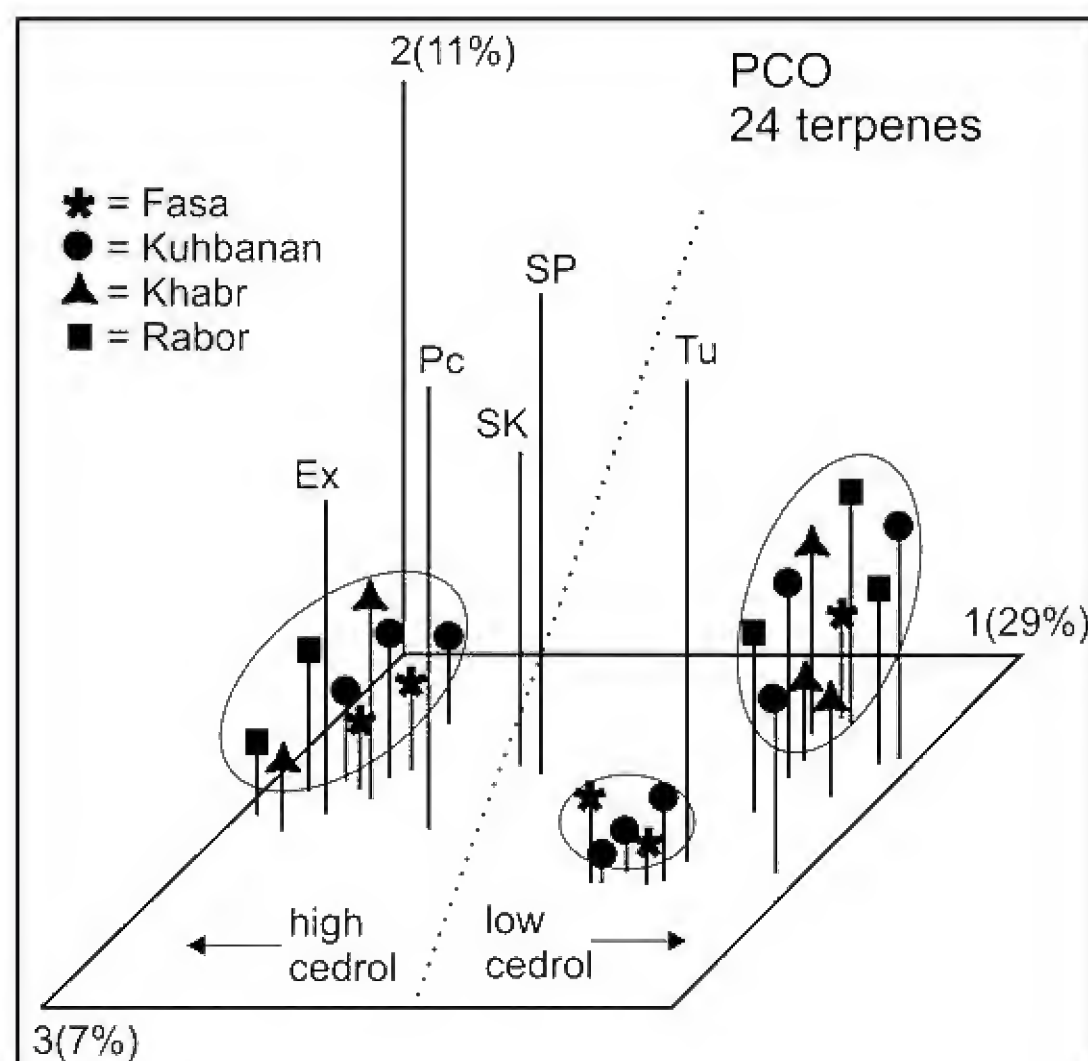


Figure 5. PCO of southern Iran *Juniperus* from 4 populations (see map, Figs. 1, 4), using 24 terpenes. The five exemplar oils are: Ex = *J. excelsa*, Turkey; Pc = *J. polycarpus*, Armenia; SK = *J. seravschanica*, Kazakhstan, SP = *J. seravschanica*, Pakistan; Tu = *J. p. var. turcomanica*, Kopet Mtns., Turkmenistan.

The low cedrol trees are loosely grouped with the *J. p. var. turcomanica* exemplar (Tu, Fig. 5). The sub-group of low cedrol oils (Fig. 5, fore-ground) is composed of 3 trees from Kuhbanan (filled circles, Fig. 5) and 2 trees from Fasa (stars, Fig. 5), with no samples from Khabr or Rabor. But the small sample numbers could have failed to include all populations in this sub-group.

The large amount of variation in the oils from each population is very likely due to hybridizing. The fact that many of samples displayed transgressive variation in several terpenoids, is suggestive of hybridization. Adams and Tsumura (2012) reviewed several papers on the inheritance of terpenes in conifers and found that terpenes seemed a little more likely to exceed the concentration (i.e., transgressive) of either parent (in a hybrid cross), than to be at intermediate levels. Hanover (1966) examined the monoterpene concentrations in 17 F₁ hybrids and their parents and found transgressive inheritance in 9/17 (α -pinene), 10/17 (β -pinene), 1/17 (δ -3-carene) and 6/17 (limonene) instances.

Cool et al. (1975), studying inheritance of terpenes in *Cupressus* hybrids, found 7/13 terpenes to be transgressive in the oils of hybrids. Adams and Stoeck (2013) analyzed the inheritance of terpenes in artificial hybrids of *Pseudotsuga menziesii* and var. *glauca*. They reported intermediate inheritance in 11/25 terpenes and transgressive inheritance in 14/15 terpenes. Adams and Tsumura (2012) analyzed artificial hybrids between cultivars of *Cryptomeria japonica* and reported intermediate inheritance for 7 terpenes and transgressive inheritance for 8 terpenes. Interestingly, the heartwood oils, cedrol, widdrol, cis-thujopsene, etc. were inherited as a group as a Mendelian dominant/recessive fashion, with a second

gene(s) as a modifier. This genetic system made the detection of hybrids very difficult; as hybrids' oils with heartwood components were nearly identical to one parent in the ordination. Removing the heartwood components from the data set aided the detection of hybrids, but a few hybrids still ordinated close to one parent.

It may be that DNA sequencing will aid in the understanding of variation in *Juniperus* in southern Iran (in progress). It seems likely that considerable field work will be needed resolve this situation.

ACKNOWLEDGEMENTS

This research was supported in part with funds from Baylor University. Thanks to Tonya Yanke for lab assistance.

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Table 1. Leaf essential oils for the multi-seeded junipers of Iran and adjacent areas. Exc - *J. excelsa*, 13193 (9433-35) Eskisehir; Poly - *J. polycarpus* var. *polycarpus*, 13194 (8761-63); SeraK - *J. seravschanica* - 13196 (8224-26); Kazakhstan, SeraP - *J. seravschanica*, 13195 (8483-85), Pakistan; Turco - *J. p.* var. *turcomanica* 13197 (8758-90); Kuh6, 13764, high cedrol, Kuhbanan; Kuh9, 13767, low cedrol, Kuhbanan, Kbr5, 13772, low cedrol, Khabr. Components in boldface were used in numerical calculations of similarities.

KI	Compound	Exc	Poly	SeraK	SeraP	Kuh6	Kuh9	Kbr5	Turco
926	tricyclene	0.2	0.1	0.1	0.1	0.1	0.2	0.2	0.1
931	α -thujene	-	t	0.3	0.4	t	t	-	t
939	α-pinene	41.7	39.9	48.3	19.9	48.7	62.5	57.6	45.0
953	α -fenchene	0.3	t	0.1	0.1	0.1	0.1	0.3	0.1
953	camphene	0.2	0.2	0.3	0.2	0.3	0.4	0.5	0.2
957	thuja-2,4(10)-diene	0.1	t	t	-	t	t	0.1	t
961	verbenene	t	t	t	t	t	t	t	t
976	sabinene	0.1	0.4	0.5	0.5	-	t	-	0.3
980	β-pinene	0.7	0.5	1.2	0.9	1.4	1.1	1.0	0.8
991	myrcene	1.2	1.3	18.3	27.4	1.9	2.4	2.4	1.9
1005	α -phellandrene	0.1	-	0.1	0.1	t	t	0.1	-
1011	δ-3-carene	5.3	t	-	0.8	1.3	0.9	3.4	t
1018	α -terpinene	0.1	t	0.1	0.1	t	t	t	t
1026	p-cymene	0.6	0.2	0.2	0.6	0.3	0.5	0.5	0.2
1031	limonene	1.2	1.0	1.8	1.4	4.3	6.3	5.2	1.0
1031	β-phellandrene	0.9	0.4	1.3	1.3	2.8	4.2	3.5	0.8
1050	(E)- β -ocimene	t	-	t	t	-	-	-	-
1062	γ -terpinene	0.5	0.3	0.7	1.0	0.3	0.5	0.4	0.4
1068	cis-sabinene hydrate	t	t	0.1	0.2	-	-	-	t
1088	terpinolene	1.1	0.6	0.9	0.8	0.5	1.0	1.5	0.8
1098	linalool*	-	0.1	0.3	0.8	3.8	2.6	0.8	t
1112	endo-fenchol	t	-	-	-	-	0.2	0.1	-
1116	3-methyl butanoate, 3-methyl-3-butenyl-	t	-	-	-	t	-	-	0.1
1121	cis-p-menth-2-en-1-ol	0.1	-	t	-	-	-	-	-
1125	α -campholenal	0.5	0.3	t	0.1	0.1	0.2	0.2	0.2
1139	trans-pinocarveol	0.8	0.2	-	t	0.3	0.3	0.3	0.2
1140	cis-verbenol	0.2	t	-	-	t	t	t	t
1143	camphor	1.2	-	t	-	-	-	-	1.0
1143	trans-verbenol	-	1.1	-	0.3	0.6	1.3	0.7	-
1148	camphene hydrate	0.1	-	t	-	t	t	t	t
1159	p-mentha-1,5-dien-8-ol	0.1	t	-	t	-	-	-	0.1
1165	borneol	-	-	0.1	-	0.3	0.1	0.3	-
1172	cis-pinocamphone	0.2	t	-	-	t	t	0.1	-
1177	terpinen-4-ol	0.1	t	0.3	0.4	-	t	-	t
1179	naphthalene	0.1	0.2	t	-	-	-	-	0.6
1183	p-cymen-8-ol	0.1	t	t	-	t	-	t	t
1189	α -terpineol	t	t	0.1	t	0.2	0.2	t	t
1193	4Z-decenal	-	-	-	-	0.3	-	0.1	-
1204	verbenone	0.2	0.2	t	t	0.1	0.2	0.1	0.1
1217	trans-carveol	0.2	t	-	-	0.1	0.2	0.1	t
1241	isoamyl hexanoate	-	-	-	-	0.1	0.4	0.1	-
1243	hexyl 3-methyl butanoate	-	0.2	t	t	-	-	-	0.4
1252	piperitone	0.1	-	-	-	-	-	-	-
1257	4Z-decen-1-ol	-	-	-	0.1	0.6	t	0.3	-
1285	bornyl acetate	0.4	0.4	0.6	0.4	0.2	0.1	0.4	0.3
1286	linalool oxide acetate (pyranoid)	0.2	-	-	-	-	-	-	-
1290	trans-sabinyl acetate	-	-	-	0.2	-	-	-	-
1319	(2E,4E)-decadienal	2.4	-	0.1	0.3	-	-	-	t

KI	Compound	Exc	Poly	SeraK	SeraP	Kuh6	Kuh9	Kbr5	Turco
1320	149,91,77,164	-	-	-	-	-	0.3	-	-
1339	δ -elemene	-	t	t	t	-	t	-	0.1
1376	α -copaene	-	-	t	t	-	-	-	0.1
1382	hexyl n-hexanoate	-	0.2	-	0.2	0.3	0.5	0.6	0.1
1383	β -bourbonene	0.1	-	-	-	-	-	-	-
1389	β -elemene	-	-	t	t	-	t	0.2	0.2
1389	β -cubebene	0.1	0.1	t	-	-	-	-	t
1409	α-cedrene	0.8	1.0	0.2	0.6	2.1	-	-	0.1
1409	1,7-di-epi- β -cedrene	0.7	1.5	0.1	0.7	-	-	-	-
1418	(E)-caryophyllene	-	0.8	0.1	0.3	-	0.3	0.4	0.6
1418	β -cedrene	0.5	0.2	0.1	0.2	0.8	-	-	t
1429	cis-thujopsene	0.3	0.4	0.2	0.2	0.3	-	-	-
1434	γ -elemene	-	-	-	-	-	0.1	0.4	t
1446	cis-muurolo-3,5-diene	0.2	-	t	-	-	-	-	t
1454	α -humulene	0.1	-	t	t	-	0.3	t	0.2
1458	(E)- β -farnesene	0.2	0.3	t	0.2	0.4	-	-	-
1461	cis-muurolo-4(14),5-diene	-	0.1	t	t	0.1	-	-	0.2
1466	β -acoradiene	0.1	0.2	-	t	-	-	-	-
1473	trans-cadina-1(6),4-diene	0.2	-	t	-	-	-	-	-
1477	γ -muurolene	-	-	t	t	-	0.3	t	0.3
1480	germacrene D	0.6	0.6	0.1	0.3	0.1	0.5	0.2	1.3
1489	β -selinene	-	-	-	-	-	t	0.2	-
1491	trans-muurolo-4(14),5-diene	0.2	-	t	0.2	-	-	-	0.2
1493	epi-cubebol	0.3	-	0.1	-	-	-	-	0.5
1494	bicyclogermacrene	-	0.3	-	t	-	-	-	-
1496	viridiflorene	-	-	-	-	-	0.3	0.3	-
1499	α -muurolene	0.1	-	0.3	0.2	-	-	-	0.7
1502	cuparene	0.1	-	-	t	-	-	-	-
1503	germacrene A	-	-	-	-	-	-	-	0.2
1509	β -bisabolene	0.1	t	-	t	0.1	-	-	-
1513	α -alaskene	0.2	0.1	-	0.3	0.1	-	-	-
1513	γ-cadinene	-	1.0	0.4	0.7	-	1.6	-	1.7
1513	cubebol	0.4	-	-	-	-	-	-	-
1521	β -sesquisphellandrene	-	-	-	-	0.2	-	-	-
1524	δ-cadinene	0.5	0.8	1.1	0.8	-	1.6	t	2.8
1532	E- γ -bisabolene	0.2	0.3	-	-	-	-	-	-
1532	γ -cuprenene	0.2	-	-	t	0.1	-	-	-
1533	sesquiterpene, <u>161</u> , <u>204</u> , 133,189	-	-	-	-	-	-	0.8	-
1538	α -cadinene	-	-	0.2	0.1	-	-	-	0.4
1545	selina-3,7(11)-diene	-	-	-	-	-	0.3	0.4	-
1549	elemol	-	0.4	0.7	0.9	0.3	0.4	1.1	0.7
1556	germacrene B	-	1.6	0.5	0.7	0.3	1.4	6.3	2.8
1574	germacrene D-4-ol	-	1.5	1.2	2.9	-	2.0	-	8.9
1587	allo-cedrol	1.9	2.3	0.8	1.2	1.1	-	-	-
1596	cedrol	25.4	30.3	13.8	22.7	23.6	-	0.1	0.2
1606	humulene epoxide II	t	-	-	-	-	-	-	-
1606	β -oplophenone	t	-	-	-	-	0.4	-	0.4
1627	1-epi-cubenol	0.5	-	-	-	-	-	-	-
1630	γ -eudesmol	-	0.4	-	-	-	-	0.4	-
1640	epi- α -cadinol	t	0.2	0.3	0.4	-	0.5	-	1.4
1640	epi- α -muurolol	t	0.3	0.4	0.4	-	0.5	-	1.4
1642	selina-3,11-dien-6- α -ol	-	-	-	-	-	-	0.2	-
1645	α -muurolol	t	t	0.1	0.1	-	t	-	0.5
1649	β-eudesmol	-	t	0.2	0.2	0.3	0.3	1.0	0.2
1652	α-eudesmol	-	0.2	0.1	0.2	0.3	0.5	1.0	t
1653	α -cadinol	t	0.5	0.9	1.0	-	0.5	-	3.6

KI	Compound	Exc	Poly	SeraK	SeraP	Kuh6	Kuh9	Kbr5	Turco
1661	sesquiterpene 85,57,41,238	1.0	-	-	-	-	-	-	t
1663	β -atlantone	0.6	-	-	-	-	-	-	-
1666	bulnesol	-	0.3	-	0.2	-	-	-	0.2
1666	(2E,4E)-decadienol	0.6	-	-	-	-	-	-	-
1688	shyobunol	-	1.2	0.7	1.6	0.3	t	0.4	2.1
1700	eudesm-7(11)-en-4-ol	-	-	-	-	-	-	0.3	-
1789	8- α -acetoxylemol	-	-	-	t	-	-	-	0.1
1961	sandaracopimara- 8(14),15-diene	t	-	-	-	-		-	-
1989	manoyl oxide	0.1	0.4	-	0.2	0.1	t	0.1	-
2054	abietatriene	0.1	t	-	t	-	-	t	t
2080	abietadiene	0.5	0.8	t	0.3	-	t	0.2	0.7
2147	abieta-8(14),13(15)-diene	-	-	-	-	-		-	t
2181	sandaracopimarinal	-	-	-	-	-		-	0.1
2288	4-epi-abietal	0.2	1.5	0.1	1.2	0.1	0.2	0.5	1.9

**Hybridization between *Vachellia collinsii* and *V. pennatula* (Fabaceae: Mimosoideae)
in the New World tropics**

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ABSTRACT

Principal component analysis (PCA) and principal coordinate analyses (PCoA) suggests that *Vachellia collinsii* and *V. pennatula* occasionally hybridize. This putative hybrid shows a relationship to *V. collinsii* with some enlarged stipular spines that are rarely inhabited by ants, some leaflets with Beltian bodies, and usually 2 to 4 enlarged and cup-shaped petiolar glands. In contrast, the putative hybrid shows a relationship to *V. pennatula* with many pinna-pairs/leaf, numerous leaflets/pinna, small leaflets, and puberulent leaves. The hybrid between *V. collinsii* and *V. pennatula* (*Vachellia* × *ziggyi*) is described. Published on-line www.phytologia.org *Phytologia* 95(4): 296-301 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: *Vachellia collinsii*, *V. pennatula*, Mimosoideae, hybridization.

The genus *Vachellia*, which includes the species of *Acacia* s.l. with paired stipular spines and flowers that lack a floral disc, consists of 60 species in the New World tropical and subtropical areas from southern United States south to Argentina (Seigler and Ebinger 2005). Also, nearly 100 additional species are found in the Old World tropics and subtropics of Asia, Africa, and Australia.

Hybrids between New World *Vachellia* species are occasionally encountered. These hybrids mostly involve species belonging to the *Vachellia macracantha* species group that includes the ant-acacias and a few related taxa (Maslin and Stirton 1997, Ebinger and Seigler 1992, Seigler and Ebinger 1995). Hybrids between ant-acacia species and between ant-acacias and non-ant-acacias have been discussed by Janzen (1974), Ebinger and Seigler (1992), and Seigler and Ebinger (1995). These studies indicate that at least four ant-acacia species hybridize with various non-ant-acacia species including *V. campechiana* (Mill.) Seigler & Ebinger, *V. macracantha* (Willd.) Seigler & Ebinger, and *V. pennatula* (Schltdl. & Cham.) Seigler & Ebinger (Ebinger and Seigler 1987, Seigler and Ebinger 1988). The present study was undertaken to examine the morphological differences of probable hybrid individuals involving the ant-acacia species *V. collinsii* (Saff.) Seigler & Ebinger and a related non-ant-acacia species, *V. pennatula*. These two species are sympatric throughout much of Mexico and Central America, and are commonly associated with disturbance in thorn-scrub and ruderal communities.

MATERIALS AND METHODS

These analyses were based on herbarium specimens of the putative parents and hybrids from Mexico and Central America (Appendix I). Initially, the specimens were separated into taxonomic groups based on overall morphological similarity. These specimens were scored for 12 characters (Appendix II). These data served as the source of characters for principal components analyses (PCA) and principal coordinates analyses (PCoA). Three or more measurements were made for each continuous character of each specimen. These values were then plotted to confirm that gaps in the data exist.

A PCA to identify groupings of the specimens examined was carried out. For this analysis, the data were first standardized and a correlation matrix, eigenvalues, and eigenvectors were calculated using NTSYS-pc version 2.1 (Rohlf 2000). Eigenvectors were scaled by the square root of λ . The axes were rotated and the resulting loading values graphically represented as both two- and three-dimensional plots.

To carry out the PCoA analysis, Gower's resemblance coefficients were calculated (Legendre and Legendre 1983, Podani 1999, Dickinson 2000). The nature of each character was designated as binary, multistate, or quantitative descriptors and all characters were weighted equally (Dickinson 2000). The data matrix was transformed by the DCENTER algorithm using distances squared and eigenvectors and eigenvalues calculated with NTSYS-pc version 2.1 (Rohlf 2000). Eigenvectors were scaled by the square root of λ . The resulting loading values were graphically represented as both two- and three-dimensional plots (Figure 1).

RESULTS

The analyses involved 11 specimens of *Vachellia collinsii*, 11 specimens of *V. pennatula* and four probable hybrids. The PCA based on 12 characters (Appendix II), and a PCoA based on Gower's similarity coefficients for species scored proved to be similar (Figure 1). In the PCA, the first three principal components accounted for 96% of the total variance. Spine size (Ssi), Beltian bodies (Bbo), leaflet length (Lle) and pinna pair number (Ppn) (characters 1, 2, 9, and 5) were most important for determining the component score of the first axis; pinna length (Ple), leaflets/pinna (Lpi), petiole gland shape (Pgs), and leaflet pubescence (Lpu) (characters 6, 7, 4, and 12) were most important for determining the second axis. The specimens used in this analysis represented distinct groupings in both PCA and PCoA, the results showing that the parent species were well separated with putative hybrids located between them (Figure 1).

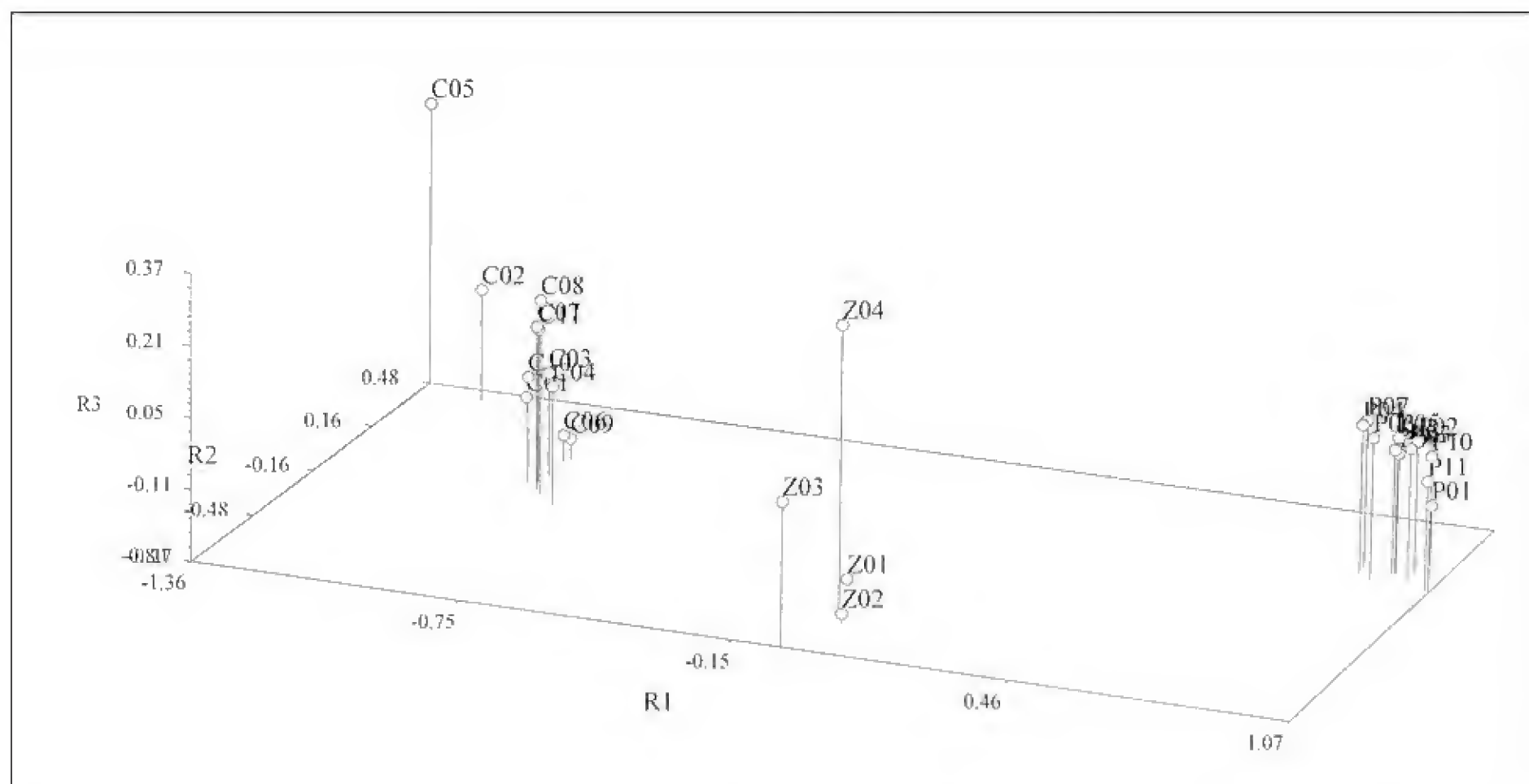


Figure 1. Plot of axis 1 v. 2 for the principal components analysis using the 12 characters (Appendix II) on 11 specimens of *Vachellia collinsii* (C01-C11), 11 specimens of *V. pennatula* (P01-P11), and four specimens of the probable hybrids (*V. x ziggyi*) (Z01-Z04).

DISCUSSION

Vachellia collinsii has the most extensive distribution of all ant-acacias, extending from central Mexico (states of Campeche, Chiapas, Durango, Guerrero, Nayarit, Oaxaca, Quintana Roo, and Yucatán), south through Central America into Colombia (states of Atlántico and Bolívar). The non-ant-acacia, *V. pennatula* has a similar, but more extensive distribution, extending from northern Mexico (states of Sonora and Tamaulipas) south through most of Mexico, through Central America, and into Colombia (state of Valle de Cauca) and Ecuador (states of Cotopaxi and Pichincha). The hybrid, in contrast, has a somewhat restricted range and appears to arise from occasional hybridization in areas where the two parental species overlap. Based on intermediacy in PCA analyses, all collections we have seen appear to be F₁-hybrids. We have only encountered specimens from Guatemala (state of Guatemala), Mexico (states of Chiapas and Oaxaca) and Nicaragua (states of Estelí and Madriz) (Appendix I).

The hybrid *Vachellia collinsii* x *V. pennatula* can easily be separated from both parent using many of the characteristics listed in Appendix I. The most obvious and commonly used characteristics include: occasional Beltian bodies on a few leaflets and occasional enlarged stipular spines on the hybrid that are absent in *V. pennatula* but common in *V. collinsii*; the presence of 2 to 5 broadly dome-shaped to volcano-shaped petiolar glands for *V. collinsii*, 2 to 4 enlarged and cup-shaped petiolar glands in the hybrid, while in *V. pennatula* the petiolar gland is solitary and flat; and leaflets that are larger in *V. collinsii* (6-13 mm long and 1.3-3.1 mm wide), smaller in the hybrid (2.0-4.5 x 0.8-1.1 mm), and much smaller in *V. pennatula* (0.8-3.0 x 0.4-0.7 mm). The proposed new hybrid is described, being named after a cartoon character affectionately called Ziggy.

Diagnosis: *Vachellia x ziggyi* Seigler & Ebinger differs from other *Vachellia* species in that it has alternate leaves 40 - 160 mm long, symmetrical stipular spines 10 - 40 x 2 - 6 mm near the base, 2-4 petiolar glands scattered along the petiole, and inflorescences densely-flowered spikes, 1.5 to 2 times longer than wide, in short racemose clusters.

***Vachellia x ziggyi* Seigler & Ebinger, *nothomorph nov.* (*Vachellia collinsii* x *V. pennatula*)**

TYPE: MEXICO, Oaxaca. A 5 km al NE de San Pedro Tepanatepec, Distr. Juchitán, 200 m, 16 Dec 1978, *M. Sousa, L. Rico & P. Basurto 10157* (holotype: MO). (Figure 2)

Shrub or small **tree** to 5 m tall; bark not seen; twigs dark reddish brown, slightly flexuous, glabrous to lightly puberulent; short shoots absent; prickles absent. **Leaves** alternate, 40–160 mm long; stipular spines light to dark purple brown, symmetrical, terete to sometimes slightly flattened, straight, stout and inflated, 10–40 x 2–6 mm near the base, glabrous to lightly puberulent, some spines not enlarged, these usually less than 5 mm long; petiole adaxially grooved, 4–10 mm long, puberulent; petiolar glands (1)2 to 4, scattered along the petiole, sessile, enlarged and cup-shaped, apex circular oblong and depressed, base 1.1–2.5 mm long, sometimes the glands overlapping and continuous, puberulent; rachis adaxially grooved, 30–150 mm long, puberulent, glands absent to sometimes present between the upper 3 to 5 pinna pairs; pinnae 6 to 20 pairs per leaf, 15–40 mm long, 4–9 mm between pinna pairs; paraphyllidia absent; petiolules 0.4–0.9 mm long; leaflets 12 to 33 pairs per pinna, 0.5–1.2 mm between leaflets, linear, 2.0–5.2 x 0.8–1.2 mm, glabrous to lightly puberulent, lateral veins not obvious, only one vein from the base, base oblique, margins lightly ciliate, apex obtuse; Beltian bodies rare, usually only on the lower few leaflets of some pinna. **Inflorescence** a densely-flowered subglobose spike about 1.5 to 2 x longer than wide, 9–15 x 6–8 mm, in short racemose clusters to 50 mm long with 1–4 spikes per node in each of the 2 to 4 nodes; peduncles 10–25 x 0.8–1.6 mm, densely puberulent; receptacle slightly enlarged; involucre 3- to 5-lobed, located just below the spike, persistent; floral bracts stalked, 1.0-1.5 mm long, apex oval, ciliate, deciduous. **Flowers** sessile, yellow; calyx 5-lobed, 0.8–1.3 mm long, glabrous to lightly puberulent; corolla 5-lobed, 1.4–1.8 mm long, lobes less than one-quarter the length of the corolla,

glabrous; stamens 40 to 60; stamen filaments 2.0–3.3 mm long, distinct; anther glands absent; ovary glabrous, sessile. **Legumes** not seen. **Seeds** not seen.

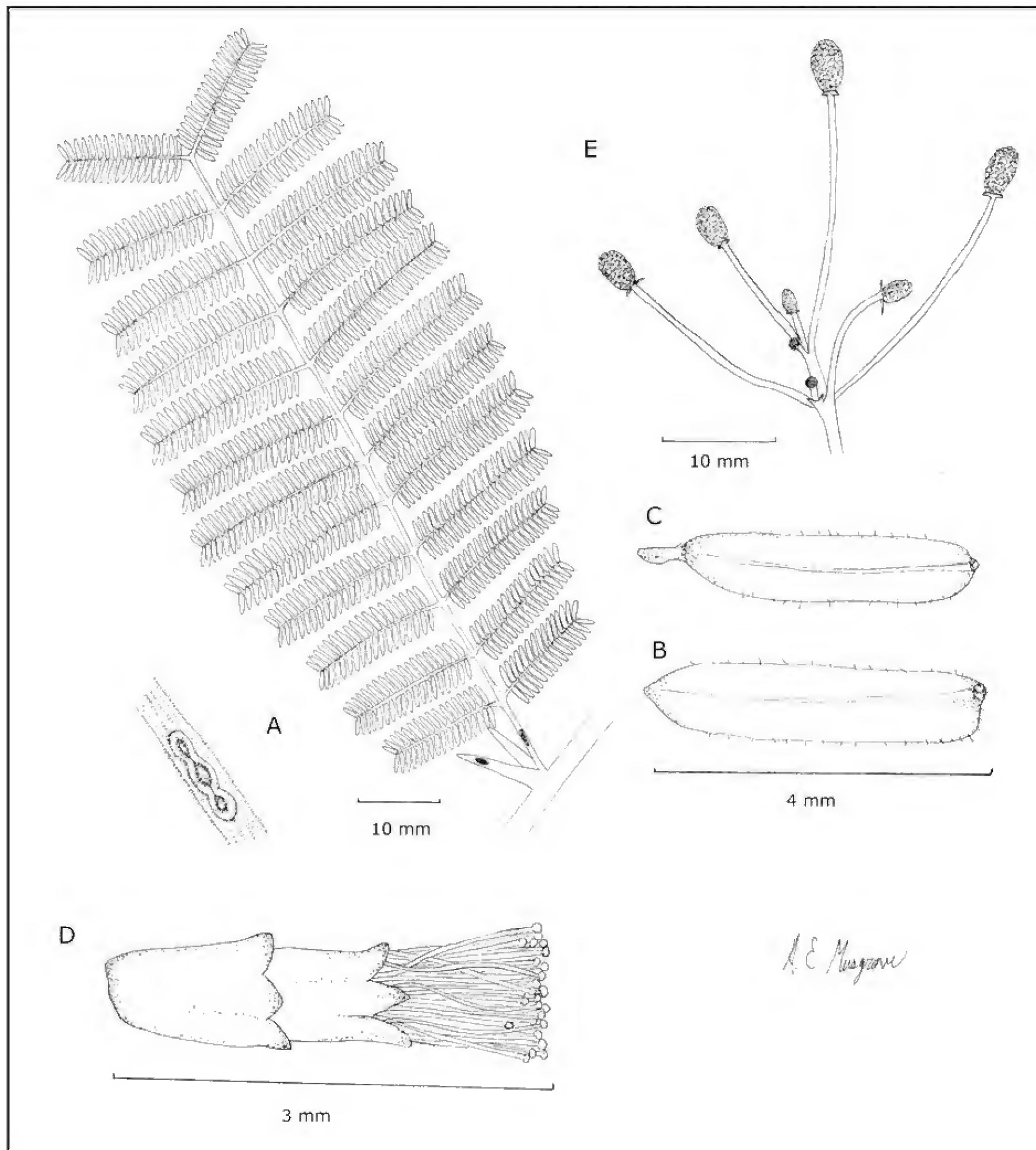


Figure 2. *Vachellia x ziggyi* Seigler & Ebinger, A: Leaf with petiolar glands and stipular spines including an entrance made by ants (Janzen 571), B: Leaflet (adaxial surface) (Janzen 571). C: Leaflet with incompletely-formed Beltian body (Janzen 571). D: Flower (Sousa et al. 10157). E: Racemose pseudo-inflorescence with immature subglobose inflorescences (Sousa & Rico 10157).

The puberulent stipular spines, petioles, and rachises of these hybrids suggest that *Vachellia pennatula* is the non-ant-acacia parent. Also, the young leaves are densely puberulent, a characteristic of young leaves of *V. pennatula*. The enlarged stipular spines, the presence of Beltian bodies on the lower 1 to 2 leaflets of some pinna pairs, and the broadly dome-shaped petiolar glands indicate *V. collinsii* as the probable ant-acacia parent. The one flowering specimen observed (*M.Sousa et al. 10157*) indicated a relationship to *V. collinsii* with subglobose inflorescences in short racemose clusters. On some herbarium specimens, the collector (Dr. Daniel H. Janzen) mentioned that *V. collinsii* was the only ant-acacia present at the site.

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APPENDIX I. Specimens examined for PCA and PCoA analyses involving *Vachellia collinsii*, *V. pennatula*, and the putative hybrid (*V. x ziggyi*).

Vachellia collinsii (Safford) Seigler & Ebinger: **COLOMBIA: Bolívar:** Arenal, 45-60 m, 22 Apr 1966, *E.Forero G. & R.Jaramillo M. 496* (EIU); Cartagena, 7 km SW of Arroyo Grande, 70 m, 31 Jul 1985, *J.L.Zarucchi & H.Cuadros 3902* (ILL). **COSTA RICA: Guanacaste:** La Pacifica, near Cañas, 28 Jul 1985, *D.S.Seigler 12269* (ILL); Finca La Pacifica, near Duck Pond, 15 Aug 1985, *D.S.Seigler 12367* (ILL). **MEXICO: Campeche:** 13 miles SW of Champotón Bridge, route 180, 5 Jun 1980, *D.S.Seigler, P.M.Richardson & S.Thompson 11606* (ILL); 13 miles SW of Champotón Bridge, route 180, 5 Jun 1980, *D.S.Seigler, P.M.Richardson & S.Thompson 11607* (ILL). **Oaxaca:** 30 km E of Puerto Escondido, route 200, 18 Jul 1993, *D.S.Seigler, D.Clarke & K.Potgieter 13930* (ILL). **Quintana Roo:** 51 miles S of Valladolid, Yucatán, route 295, 3 Jun 2005, *D.S.Seigler & B.R.Maslin 16038* (ILL). **Yucatán:** Chichén Itzá, 4 Jun 1980, *D.S.Seigler, R.M.Richardson & S.Thompson 11599* (ILL). **PANAMA: Panama:** 1 km E of Chorrera City limits. 30 May 1977, *J.P.Folsom 3464* (ILL). **Los Santos:** Vicinity of Tonosí, 26 Feb 1963, *W.L.Stern, R.H.Eyde & E.S.Ayensu 1831* (ILL).

Vachellia pennatula (Schltdl. & Cham.) Seigler & Ebinger: **GUATEMALA: Guatemala:** 19 km S of Guatemala City, route 8, 1 Sep 1965, *D.H.Janzen* 776 (ILL). **MEXICO: Morelos:** 3 km N of Alpuyeca, route 95, 19 Nov 1985, *D.S.Seigler & J.E.Ebinger* 12607 (ILL). **Jalisco:** 12 miles NW of Tecolotlán, route 80, 21 Jul 1975, *D.S.Seigler & G.Holstein* 9533 (ILL). **Oaxaca:** 39 miles W of Tehuantepec, route 190, 8 Aug 1966, *D.H.Janzen* 605 (ILL); 46.7 miles W of Tehuantepec, route 190, 10 Jan 1965, *D.H.Janzen* 757 (ILL); 10 km SE of Matatlán, route 190, 19 Jul 1993, *D.S.Seigler, H.D.Clarke & K.Potgieter* 13955 (ILL); 11 miles SE of Huahuapan de León, route 180, 28 May 2005, *D.S.Seigler, J.Miller & B.R.Maslin* 15989 (ILL); **Puebla:** Near Izúcar de Matamoros, route 160, 5 Jul 1986, *D.S.Seigler & B.Maslin* 12685 (ILL). **Veracruz:** 12 miles W of Conejos on road to Huatusco, 26 Apr 1964, *D.H.Janzen* 723 (EIU); 1 mile E of Actopan, route 140, 18 Nov 1985, *D.S.Seigler & J.E.Ebinger* 12540 (EIU); 23.4 miles W of El Tamarindo, route 140, 16 Sep 1984, *D.S.Seigler, S.Berlocher & D.Nickrent* 12231 (ILL).

Vachellia x ziggyi Seigler & Ebinger: **GUATEMALA: Guatemala:** 16.2 miles NE of Guatemala City, road to Puerto Barrios, 14 May 1965, *D.H.Janzen* 744 (EIU, ILL), 745 (EIU, MO), 746 (EIU, MO), 747 (EIU, MO). **MEXICO: Chiapas:** Suckers from cut stump, pasture, 8.5 miles S of La Trinitaria, route 190, 1160 m, 7 Aug 1966, *D.H.Janzen* 572 (EIU), 573 (EIU), 574 (EIU). **NICARAGUA: Estelí:** 15.8 miles W of Sebaco, 550 m, 11 May 1976, *D.H.Janzen* 742 (EIU). **Madriz:** 2.5 miles SW of Somoto, 11 May 1965, *D.H.Janzen* 737 (EIU), 738 (EIU).

APPENDIX II. Characters scored for principal component (PCA) and principal coordinate analyses (PCoA) for the *Vachellia collinsii/pennatula* complex.

1. Spine size (Ssi) 1 = enlarged, mostly inhabited by ants, 2 = slightly enlarged, mostly not inhabited by ants, 3 = not enlarged nor inhabited by ants.
2. Beltian bodies (Bbo) 1 = present on many leaflets, 2 = restricted to a few leaflets on lower pinna, 3 = absent.
3. Petiole gland number (Pgn) 1 = glands 2-5 per petiole, 2 = solitary.
4. Petiole gland shape (Pgs) 1 = domed-shaped; 2 = flattened.
5. Pinna pair number (Ppn).
6. Pinna length near middle of leaf (mm) (Ple).
7. Leaflets/pinna (Lpi).
8. Leaflets, distance between (mm) (Ldb).
9. Leaflet length (mm) (Lle).
10. Leaflet width (mm) (Lwi).
11. Leaflet venation (Lve) 1 = lateral veins obvious, 2 = lateral veins not obvious.
12. Leaflet pubescence (Lpu) 1 = glabrous or nearly so, 2 = puberulent.

The volatile leaf oils of three *Juniperus communis* varieties from Bulgaria

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ABSTRACT

The compositions of the leaf essential oils of *Juniperus communis*, *J. c. var. sibirica* (*J. communis* var. *saxatilis* Pall.) and *J. c. var. pygmaea* (*J. c. var. saxatilis*) from Bulgaria are reported and compared with *J. communis* (Sweden) and *J. c. var. saxatilis* (Switzerland). The leaf volatile essential oils of *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria are high in α -pinene (21.4 - 38.4%), sabinene (10.5 - 19.6%), limonene (1.8 - 5.5%), β -phellandrene (2.7 - 8.3%) and terpinen-4-ol (3.2 - 7.5%). PCO revealed some clustering of the *J. sibirica* samples, but most of the samples were interspersed. It seems likely that hybridization is occurring and, if so, could explain these results. At the present time, *J. pygmaea* seems to be a shrubby form of *J. communis* and *J. sibirica* appears to be conspecific with *J. c. var. saxatilis*. Published on-line www.phytologia.org *Phytologia* 95(4): 302-307 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: *Juniperus communis*, *J. c. var. sibirica*, *J. c. var. pygmaea*, Bulgaria, leaf terpenes.

The Flora of Bulgaria (Dimitrov, 2002) lists 6 native *Juniperus* species in Bulgaria: *J. communis*, *J. excelsa*, *J. oxycedrus*, *J. pygmaea*, *J. sabina* and *J. sibirica*. Adams and Tashev (2012) reported that *J. oxycedrus* from Bulgaria is actually *J. deltoides* that grows from Italy eastward through Turkey. Of interest to the present work, are the resolution and taxonomy of *J. communis*, *J. pygmaea* and *J. sibirica* (the latter two taxa treated as *J. c. var. saxatilis* by Adams, 2011 and Farjon, 2005, 2010). Of these 3 taxa, *J. communis* var. *communis*, grows as a small tree, whereas *J. pygmaea* and *J. sibirica* are small to spreading shrubs. They differ in their leaf morphology (Fig. 1), with *J. pygmaea* leaves being very

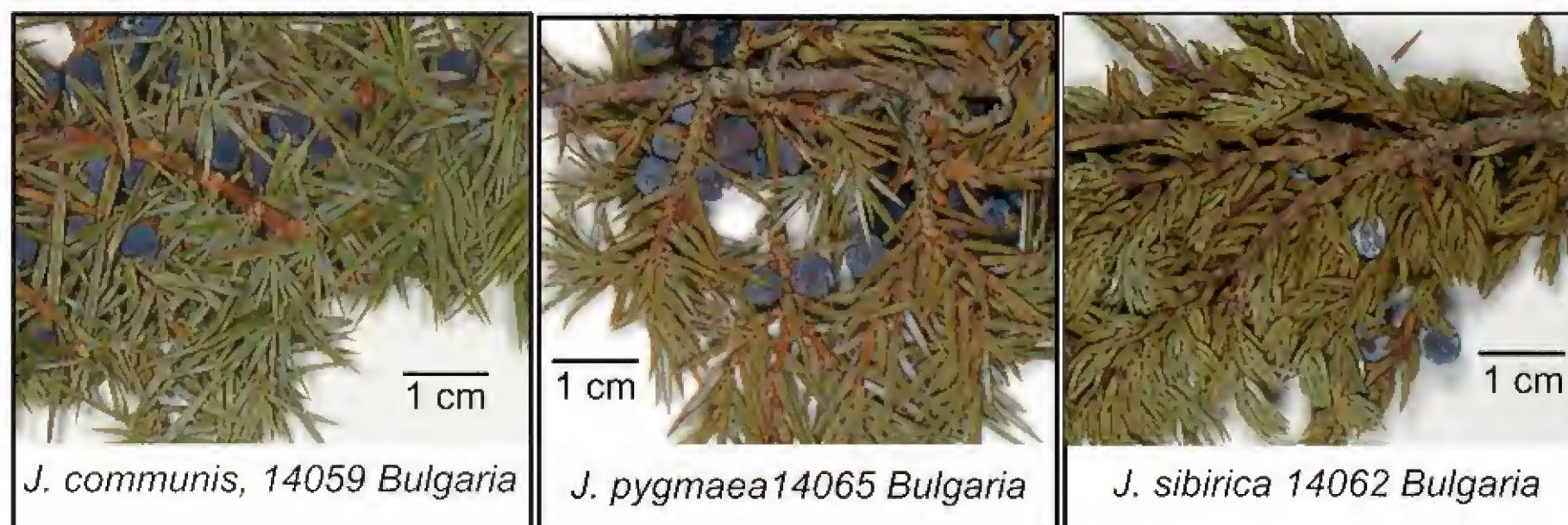


Figure 1. *J. communis*, *J. pygmaea* and *J. sibirica* specimens from Bulgaria.

similar to *J. communis*. *J. sibirica* leaves differ by being shorter, curved and more appressed to the stem (Fig. 1). In fact, the Bulgarian *J. sibirica* leaves are quite similar to *J. communis* var. *saxicola* (Fig. 2, Mongolia), but more appressed to the stem than in the specimen from Mongolia (Fig. 2).

The leaf oils of *J. communis* have been extensively studied; see Adams et al. (2010) and Adams (2013) for a review of the literature. Most recently, Lohani et al. (2013) reported the major components of *J. c.* var. *saxatilis* from alpine India to be α -pinene (31.8-49.5%), limonene (13.7 - 19.5%) and δ -3-carene (9.7 - 14.7%).

The purpose of the present study was to compare the leaf volatile essential oils from *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria to determine if their oils differ.



Fig. 2. Specimen of *J. c.* var. *saxatilis* from Mongolia.

Plant material - Bulgaria, *J. communis* var. *communis*, Adams 13730-31, 14058-60, Alex Tashev, 2012-JC1-5, Eastern Rhodopes, in protected site "Gumurdjinsky Shezhnik", locality "Madzharsky Kidik". On limestone rocks above the upper border of a forest of *Fagus sylvatica* ssp. *moesiaca*, 41° 14' 44.7" N; 25° 15' 31.9" E. elev. 1270 m.

J. pygmaea K. Koch (cf. *J. communis* var. *saxatilis* Pall.), Adams 13734-35, 14064-66, Alex Tashev, 2012-JP1-5, Central Rhodopes. Mursalitza part, locality "Piramidata". On high-mountain meadow, on a limestone rock near a forest of *Pinus sylvestris* together with *Picea abies*, 41° 40' 22.8" N; 24° 26' 36.6" E. elev. 1756 m.

Juniperus sibirica Burgsd. (cf. *J. communis* var. *saxatilis*), Adams 13732-33, 14061-63, Alex Tashev, 2012-JS11-5, Vitosha Region. Nature Park "Vitosha". Above the hut "Aleco" near the alpine timber line formed by a forest of *Picea abies*. On silicate rock together with *Vaccinium myrtillus*, *V. uliginosum*, *Ribes petraeum*, *Rubus idaeus*, *Calamagrostis arundinaceae*, *Festuca valida* (Bulgarian endemic), 42° 34' 52.1" N; 23° 17' 28.0" E. elev. 1848 m.

Exemplar specimens: *J. communis* var. *communis*, Stockholm, Sweden, Adams 8167 (7846-7848); *J. communis* var. *saxatilis*, Switzerland, Adams 11164 (7618-7621). Voucher specimens deposited in the Herbarium, Baylor University (BAYLU).

Fresh or air dried (100 g) leaves were steam distilled for 2 h using a circulatory Clevenger-type apparatus (Adams, 1991). The oil samples were concentrated (diethyl ether trap removed) with nitrogen and the samples stored at -20° C until analyzed. The extracted leaves were oven dried (48h, 100° C) for the determination of oil yields. The oils were analyzed on a HP5971 MSD mass spectrometer, scan time 1/ sec., directly coupled to a HP 5890 gas chromatograph, using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column (see Adams, 2007 for operating details). Identifications were made by library searches of our volatile oil library (Adams, 2007), using the HP Chemstation library search routines, coupled with retention time data of authentic reference compounds. Quantitation was by FID on an HP 5890 gas chromatograph using a J & W DB-5, 0.26 mm x 30 m, 0.25 micron coating thickness, fused silica capillary column using the HP Chemstation software.

Terpenoids (as per cent total oil) were coded and compared among the species by the Gower metric (1971). Principal coordinate analysis was performed by factoring the associational matrix using

the formulation of Gower (1966) and Veldman (1967). Principal components analysis (PCA) follows the formulation of Veldman (1967).

RESULTS AND DISCUSSION

The leaf volatile essential oils (Table 1) of *J. communis*, *J. pygmaea* and *J. sibirica* from Bulgaria are high in α -pinene (21.4 - 38.4%), sabinene (10.5 - 19.6%), limonene (1.8 - 5.5%), β -phellandrene (2.7 - 8.3%) and terpinen-4-ol (3.2 - 7.5%). They are noticeably different from *J. communis* (Sweden) and *J. c. var. saxatilis* (Switzerland) in α -pinene and sabinene. However, α -pinene and sabinene are known to vary considerable geographically (Filipowicz et al., 2006, Adams et al., 2010). Filipowicz et al. (2006) reported low-sabinene chemotypes with 0.0 - 2.12% sabinene and high-sabinene chemotypes with 25.6 - 55.3% sabinene were interspersed between *J. communis* from lowlands and *J. nana* (= *J. communis* var. *saxatilis*) from high elevation. About 85% of the low-(to medium) sabinene plants were *J. communis* and about 50% of the *J. nana* plants had amounts of high-sabinene. Interestingly, *J. communis* (low elevation, Sweden) had 0.7% sabinene and *J. c. var. saxatilis* (high elevation, Switzerland) had 32.8% sabinene (Table 1). However, the Bulgarian samples do not follow this trend, but have the lowest sabinene (10.5%) from the highest elevation (1848m, Table 1) and the highest sabinene (19.6%) from the lowest elevation (1200m, Table 1).

To view the over-all trend among samples, similarities were computed among samples and the exemplars, *J. communis*, Sweden and *J. c. var. saxatilis*, Switzerland using 26 terpenoids. PCO of the similarity matrix resulted in six eigenroots before they began to asymptote. These six eigenroots accounted for 25.5, 14.1, 10.0, 7.7 and 6.6% of the variance among samples (OTUs). Ordination shows that most of the samples are not grouped (Fig. 3). There is some clustering of the *pygmaea* samples on the lower left quadrant (Fig. 3). Samples of *J. communis* and *J. sibirica* (*J. c. var. saxatilis*) are interspersed (Fig. 3).

It is very likely that hybridization is occurring between all three taxa. If so, this could lead to an ordination as seen in Fig. 3.

At the present time, *J. pygmaea* seems to be a shrubby form of *J. communis* and *J. sibirica* appears to be conspecific with *J. c. var. saxatilis*. Additional research will be needed to clarify the situation.

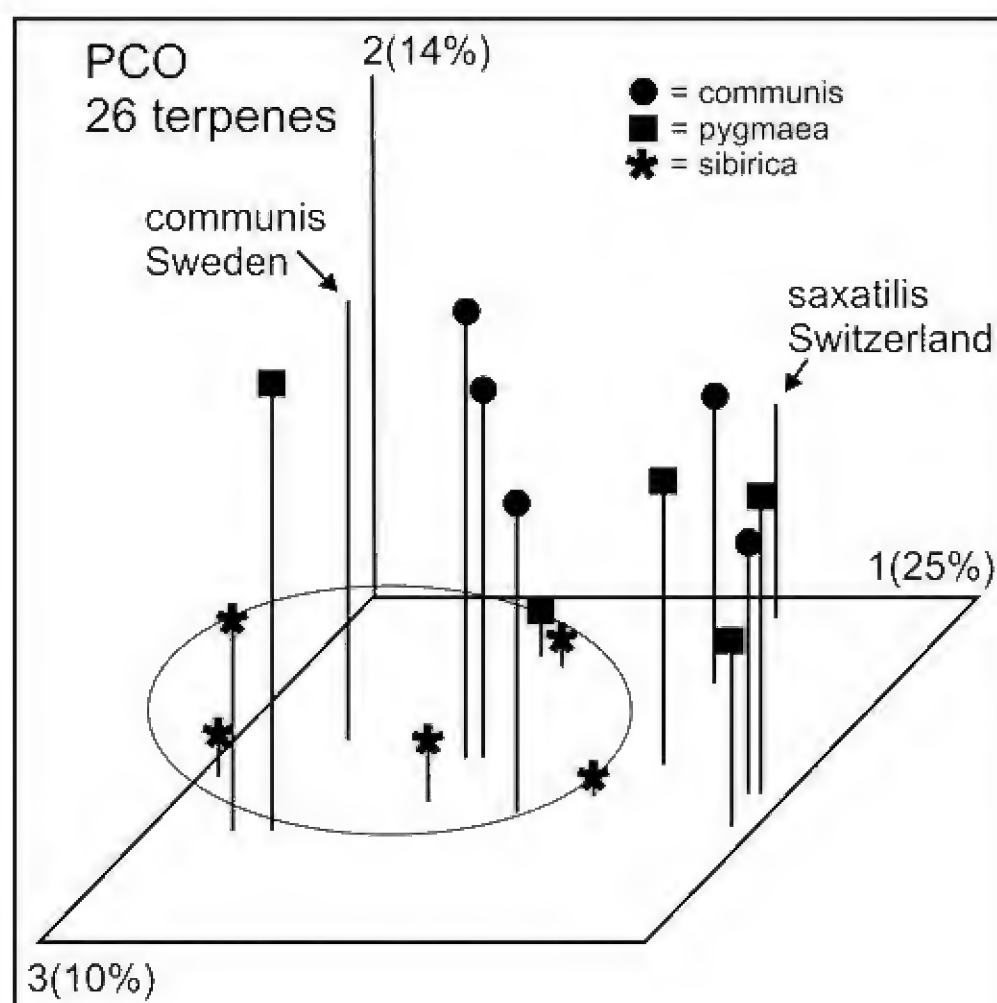


Figure 3. PCO based on 26 terpenes. see text for discussion.

ACKNOWLEDGEMENTS

This research supported in part by funds from Baylor University. Thanks to Tonnie Yanke for lab assistance.

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Table 1. Comparison of the leaf oils of *J. communis* from Bulgaria. comm Bulg - *J. communis* var. *communis*, Bulgaria; com sib - *J. sibirica*, Bulgaria; com pyg = *J. pygmaea*, Bulgaria; saxit = *J. c.* var. *saxatilis*, Switzerland; comm Swed = *J. c.* var. *communis*, Stockholm, Sweden. saxit Switz and comm Swed data from Adams et al. (2010). Components in boldface were used in numerical analyses.

KI	Compound	com Bulg 1200m	pyg Bulg 1756m	sib Bulg 1848m	saxit Switz	comm Swed
846	(E)-2-hexenal	0.5	0.7	0.5	1.2	0.7
921	tricyclene	t	t	t	t	0.3
924	α -thujene	1.9	1.9	1.0	4.1	0.1
932	α-pinene	26.7	21.4	38.4	14.1	56.8
945	α -fenchene	t	t	t	0.1	0.3
946	camphene	0.2	0.2	0.3	0.2	0.6
961	verbenene	-	t	0.4		
969	sabinene	19.6	16.3	10.5	32.8	0.7
974	β-pinene	2.0	1.4	2.1	1.9	4.4
988	myrcene	3.5	3.4	3.1	5.0	5.2
1001	δ -2-carene	0.1	0.2	t	0.4	0.2
1002	α-phellandrene	1.0	1.5	0.4	0.5	2.1
1008	δ -3-carene	0.8	0.9	0.9	0.5	4.7
1014	α-terpinene	1.6	1.9	0.9	1.9	t
1020	p-cymene	1.4	2.1	0.5	0.3	0.3
1024	limonene	5.5	4.3	1.8	6.7	5.1
1025	β-phellandrene	8.3	6.5	2.7	0.6	8.9
1044	(E)- β -ocimene	0.2	0.3	t	0.1	0.1
1049	pentyl isobutyrate	-	-	-	-	0.2
1054	γ-terpinene	2.8	3.4	1.7	3.4	t
1065	cis-sabinene hydrate	0.8	1.3	0.4	1.8	t
1086	terpinolene	2.0	2.2	1.4	3.0	1.1
1095	linalool	0.5	0.8	0.3	-	0.1
1098	trans-sabinene hydrate	0.5	0.9	0.4	1.3	-
1100	n-nonanal	t	-	-	-	-
1103	isoamyl-isovalerate	-	-	-	t	0.1
1112	3-me-3-butenyl-isovalerate	-	-	-	-	t
1112	trans-thujone (= β -thujone)	0.2	0.2	0.1	0.6	-
1118	cis-p-menth-2-en-1-ol	0.4	0.6	0.2	-	t
1122	α -campholenal	0.3	0.5	0.1	-	t
1135	trans-pinocarveol	0.7	0.9	0.2	-	-
1140	trans-verbenol	0.3	0.9	0.2	-	-
1147	3-me-2-butenyl-isovalerate	-	-	-	-	t
1154	sabina ketone	0.3	0.3	t	-	-
1165	borneol	0.4	0.6	0.6	t	0.2
1166	p-mentha-1,5-dien-8-ol	-	-	-	-	t
1174	terpinen-4-ol	5.2	7.5	3.2	7.3	0.2
1179	p-cymen-8-ol	0.5	0.7	0.1	t	t
1179	naphthalene	-	-	-	0.3	t
1186	α-terpineol	0.8	2.5	0.5	0.4	0.2
1194	myrtenol	0.2	0.5	0.8	-	-
1204	verbenone	0.2	0.2	0.1	-	t
1207	trans-piperitol	0.1	0.2	t	-	-
1215	trans-carveol	0.3	0.3	t	-	-

1223	citronellol	0.3	0.5	0.2	-	-
KI	Compound	com Bulg	pyg Bulg	sib Bulg	saxit Switz	comm Swed
1232	thymol, methyl ether	0.2	t	0.4	0.1	-
1249	piperitone	-	-	-	-	t
1257	methyl citronellate	-	-	-	-	t
1267	(E)-cinnamaldehyde	0.5	1.3	0.3	-	-
1283	α-terpinen-7-al	0.3	0.6	0.2	-	-
1285	bornyl acetate	0.3	0.3	0.6	0.2	0.9
1324	myrtenyl acetate	0.2	0.1	0.7	-	t
1346	α -terpinyl acetate	0.1	t	0.4	0.5	-
1350	citronellyl acetate	-	-	-	-	t
1374	α -copaene	0.2	0.5	0.3	-	-
1385	trans-myrtanyl acetate	-	-	-	t	-
1389	β -elemene	0.2	0.1	0.5	t	0.2
1417	(E)-caryophyllene	0.5	0.4	0.4	t	0.7
1452	α -humulene	0.4	0.4	0.3	t	0.5
1478	γ -muurolene	t	t	0.3	t	t
1480	germacrene D	0.6	1.4	0.7	0.4	0.7
1492	cis- β -guaiene	0.2	0.1	0.5	-	-
1493	epi-cubebol	-	-	-	-	t
1500	α -muurolene	0.3	0.1	0.5	0.2	0.2
1508	germacrene A	0.1	t	0.3	0.2	0.1
1513	γ -cadinene	0.5	0.8	0.9	0.4	0.2
1522	δ-cadinene	0.8	0.9	2.1	0.8	0.5
1537	α -cadinene	t	t	0.3	t	t
1548	elemol	t	t	0.3	-	t
1559	germacrene B	t	0.4	0.6	0.3	0.3
1561	(E)-nerolidol	0.5	0.1	0.3	-	-
1574	germacrene D-4-ol	0.7	0.2	1.2	1.8	0.8
1577	spathulenol	0.1	0.6	0.2	-	t
1582	caryophyllene oxide	0.6	0.8	0.1	-	t
1606	humulene epoxide II	0.4	0.5	0.3	-	t
1627	1-epi-cubenol	0.1	0.1	0.3	t	t
1638	epi- α -cadinol	0.3	0.3	0.6	0.5	t
1639	epi- α -muurolol	0.3	0.4	0.6	0.5	0.4
1644	α -muurolol	0.1	t	0.2	0.1	t
1652	α-cadinol	0.7	0.5	1.4	1.3	0.5
1685	germacra-4(15),5,10(14)- trien-1-al	t	0.2	t	-	t
1688	shyobunol	0.1	0.1	t	-	0.7
1722	(2E,6Z)-farnesol	t	0.3	t	-	-
1987	manoyl oxide	t	0.3	0.5	0.1	-
2055	abietatriene	t	0.4	0.9	0.2	-
2087	abietadiene	t	0.3	1.4	0.4	-
2105	isoabienol	0.2	0.1	5.0	0.1	-
2314	trans-totarol	t	0.6	2.2	-	-
2370	trans-ferruginol	t	0.1	0.3	-	-

KI = Kovat's Index on DB-5(= SE54) column. Compositional values less than 0.1% are denoted as traces (t). Unidentified components less than 0.5% are not reported.

Recension of the genus *Fendlerella* (Hydrangeaceae)

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ABSTRACT

The basic taxonomy of *Fendlerella* is reviewed. The genus is comprised of four species: *F. utahensis*, with two allopatric, intergrading varieties, var. *utahensis*, of western USA, and var. *cymosa*, of southwestern USA and northern Mexico; *F. lasiopetala*, of north-central Mexico; *F. mexicana* of Pueblo, Mexico; and the newly described ***F. queretarana* B.L. Turner, sp. nov.**, from the state of Queretaro, Mexico. A key to the taxa is provided, along with maps showing their distribution. Published on-line www.phytologia.org *Phytologia* 95(4): 308-313 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Hydrangeaceae, *Fendlerella*, *F. utahensis*, *F. lasiopetala*, *F. mexicana*, *Fendlera*, *Whipplea*

The genus *Fendlerella* was erected by Heller in 1898 with the description of *F. utahensis* from collections made by Mrs. E. P. Thompson in Kanab, Kane Co., Utah, in the year 1872. The taxon was originally described as belonging to the genus *Whipplea*, but Heller aptly remarked, "That this plant is not a *Whipplea* is evident, neither does it agree much better with the genus *Fendlera*, with its large usually solitary flowers, tetramerous, and its ovoid capsule, which is attached to the calyx tube only at the base." Indeed, *Whipplea*, *Fendlera* and *Fendlerella* form a closely related triad within the Hydrangeaceae, which is well supported by morphological and DNA studies (Soltis, D.E., Q. Xiang and Hufford, 1995; Turner, 2001).

Subsequent workers added to *Fendlerella* the species, *F. cymosa* Greene ex Woot. & Standl. (1913), this reduced to a variety of *F. utahensis* by Kearney & Peebles. Three Mexican species were subsequently described, *F. mexicana* Brandegees (in 1908); *F. lasiopetala* Standl. (in 1920); and the newly described *F. queretarana* B.L. Turner, below.

The following is a Key to the closely related genera *Whipplea*, *Fendlera* and *Fendlerella*, as abstracted from Small and Rydberg (1916):

- 1. Capsule spheroidal; style deciduous, the capsule beakless.....**Whipplea**
- 1. Capsule conic to ovoid; style persistent, the capsule beaked...(2)
- 2. Sepals and petals 5; anthers w/o appendages.....**Fendlerella**
- 2. Sepals and petals 4; anthers with apical appendages.....**Fendlera**

WHIPPLEA Torr.

The genus contains but a single species, *W. modesta* Torr., largely confined to the Pacific Northwest (California, Oregon, Washington).

FENDLEREA Engelm. & Gray

The genus contains five species, as treated by Turner (2001).

FENDLERELLA Heller

Divaricately, much-branched shrubs 0.4-1.0 m high. Stems woody, strigose, the internodes much shortened. Leaves, simple, elliptic, lanceolate to oblanceolate, margins entire, often revolute, mostly 1-2

cm long, markedly strigose or not, the undersurfaces variously pubescent, 3-nervate. Flowers, inconspicuous, arranged in terminal cymose clusters. Petals small, linear to oblanceolate, white, glabrous or pubescent. Hypanthium, turbinate, ca 1.5 mm high. Capsule ca twice as long as the calyx. Base chromosome number, $x = 13$.

As treated here, a genus of four species, one of these possessing two varieties, as follows:

Key to taxa

1. Leaves sparsely pubescent, not at all bicolored.....**F. utahensis**
1. Leaves densely pubescent above and below, markedly bicolored;
plants of Mexico...(2)
2. Undersurfaces of leaves with a single layer of appressed, straight hairs;
Que.....**F. queretarana**
2. Undersurfaces of leaves pubescent with two layers, a dense lower level of
crinkly hairs and an upper layer of appressed, straight hairs; Coa, Nue, Pue...(3)
3. Upper surface of petiole markedly tufted with hairs 2-3 mm long;
leaves weakly 3-nerved beneath ; petals glabrous; hypanthium
weakly pubescent; Pue.....**F. mexicana**
3. Upper surface of petiole weakly tufted with hairs ca 1 mm long;
leaves strongly 3-nervate beneath throughout; petals usually pubescent;
hypanthium markedly pubescent.....**F. lasiopetala**

FENDLERELLA LASIOPETALA Standl., Proc. Biol. Soc. Washington 33: 67. 1920.

TYPE: **MEXICO. COAHUILA:** San Lorenzo Canyon, SE of Saltillo, 16 Apr 1905, *E. Palmer* 635 (Holotype: US).

Coa and Nue, calcareous or gypsum substrates, 1200-2200 m; flowering: Jul-Oct. **Fig. 2**
Shrubs or shrublets, 0.4-1.0 m high; stems woody, the internodes much shortened; leaves ovate to ovate-lanceolate, mostly 1.0-1.5 cm long, 0.3-0.6 cm wide, more or less bicolored, pubescent above with appressed, straight, basally enlarged hairs, below with an upper array of linear, appressed, hairs, beneath this a dense cottony layer of crinkly hairs; petals linear, white, 2-3 mm long, glabrous or pubescent with elongate white hairs; hypanthium pubescent, the lobes ca 1 mm long.

According to its author, this taxon differs from most other species in the genus in having pubescent petals, but that is a variable character, plants from the same population may possess glabrous or pubescent petals, some of these more so, some less so.

The species is represented by numerous collections at LL-TEX (Fig. 2).

FENDLERELLA MEXICANA Brandege, Zoe 5: 246. 1908.

TYPE: **MEXICO. PUEBLA:** vicinity of San Luis Tultitlanapa, "Rocky slopes. Cerro de Paxtle." July 1907, *C. A. Purpus* 2588 (Holotype: UC; isotype: MO!).

Pue, mostly calcareous substrates, 1600-2600 m; flowering: Feb-Jun. **Fig. 2**
Sub-shrubs to 1 m (?) high. **Leaves** opposite throughout, markedly bicolored; petioles 1-2 mm long, their upper surfaces markedly pubescent with tufted hairs 2-3 mm long; blades mostly elliptic to ovoid, 8-20 mm long, 3-8 mm wide, weakly 3-nerved beneath, the upper surfaces moderately pubescent with appressed hairs, the lower surfaces with an upper layer of elongate hairs, beneath these an understory of cottony, densely congested, crinkly, hairs. **Cymes** dense, terminal, ca 1.5 cm long, and as wide. **Hypanthium** turbinate, ca 2 mm long, sparsely pubescent; lobes ca 1 mm long. **Petals** white or cream, spatulate, 3-4 mm long, 1-2 mm wide, glabrous. **Capsules** 2-loculate, turbinate, ca 3 mm long, 1 mm wide.

This species is known to me only by the type, the above description largely taken from an isotype at MO.

Perez-Calix (2004) treated material from Queretaro as belonging to this taxon, but I treat such plants as constituting the newly described *F. queretarana*, below; *F. mexicana*, in addition to the pubescence mentioned in the above key, has more pronounced petals, and more prominently tufted petioles, not to mention its geographical disjunction.

FENDLERELLA QUERETARANA B.L. Turner, **sp. nov.** **Fig. 1**

n Que, calcareous soils, oak and *Juniperus-Pinus cembroides* woodlands, 1650-2600 m; Feb-Jun.

Resembling *Fendlerella mexicana* but the under surfaces of leaves pubescent with but a single layer of elongate hairs (vs 2-layered), the petals smaller (1-2 mm long, vs 2-3 mm), and the tuft of hairs at the base of petiole shorter, less pronounced (hairs ca 1 mm long, vs 2 mm).

Shrubs or shrublets, 0.5-1.0 m high. **Leaves** lanceolate, 1-2 cm long, weakly bicolored, 3-nervate beneath, if at all, the upper surfaces moderately appressed-pubescent with broad-based white hairs ca 1 mm long, lower surfaces more densely pubescent with a single layer of longer hairs ca 2 mm long; apices acute-apiculate; petioles with a basal tuft of axillary hairs ca 1 mm long. **Cymes** 10-20 flowered, arranged in terminal, aggregations, 5 mm high, ca 10 mm across. **Hypanthium**, ca 1 mm high, sparsely pubescent; sepals ovate, ca 1 mm long; petals white, linear, glabrous, 1-2 mm long, ca 1 mm wide. **Capsules** 2-loculate, ca 3 mm long. Seeds not observed.

TYPE: MEXICO. QUERETARO: Mpio. Pinal de Amoles, “1-2 km al N del Puerto del Tejamanil,” pine-oak woodlands, 2480 m, 11 Jun 1991, *E. Carranza 3187* (Holotype: TEX).

The above description was largely taken from type material. Perez-Calix (2004), in his treatment of *F. mexicana* for the Flora de Bajío, provides a more enlarged description, this based upon 11 sheets from 3 additional Municipios of Queretaro, these included in my Fig. 2.

FENDLERELLA UTAHENSIS (S. Wats.) Heller, Bull. Torrey Bot. Club 25: 626. 1898.

Fendleria utahensis Greene

Whipplea utahensis S. Wats.

As treated here, two geomorphological population systems are recognized (Fig. 3); occasional plants from one or the other complex will have intermediate characters, hence their treatment as varieties.

Key to varieties

1. Larger leaves mostly lanceolate to narrowly oblanceolate,
1.5-2.0 cm long, having apices narrowly acute.....var. **cymosa**
1. Larger leaves mostly ovate to obovate, 0.8-1.5 cm long, having
apices broadly acute to obtuse, or rounded.....var. **utahensis**

var. **utahensis**

TYPE: USA. Utah: Kane Co., Kanab on “dry rocky cliffs; July, August.” *E.T. Thompson s.n.*, probably in 1872 (Holotype GH).

This taxon is well described by numerous authors (e.g., Welsh et al., 1987) and need not be elaborated upon here. It is widely distributed in the western USA (**Fig. 3**).

var. **cymosa** (Greene) Kearney & Peebles, J. Wash. Acad. Sci. 29: 480. 1939.

Fendlerella cymosa Greene

TYPE: USA. ARIZONA: Cochise Co.: Huachuca Mts., 7 Jul 1884, *Pringle 699* (Holotype: US).

The var. *cymosa* is largely restricted to limestone boulders and rocky ledges in the southwestern USA. In Mexico it mostly occurs on calcareous or gypsum slopes in pine woodlands. Leaf size and shape is very variable in the collections from Nuevo Leon, Mexico, encompassing the range of leaf shapes and sizes found in both varieties of the species elsewhere. **Fig. 3**

The taxon was well described by Greene in his original description and by subsequent authors, and such need not be reiterated here.

Vines (1960) provided an excellent description and sketch, commenting that the taxon “has been named the Arizona Fendlerella” and correctly noted that it has narrower and more pointed leaves than the typical variety.

ACKNOWLEDGEMENTS

I am grateful to my editorial assistant, Jana Kos for helpful suggestions, and to the Missouri Botanical Garden (MO), for the loan of type material.

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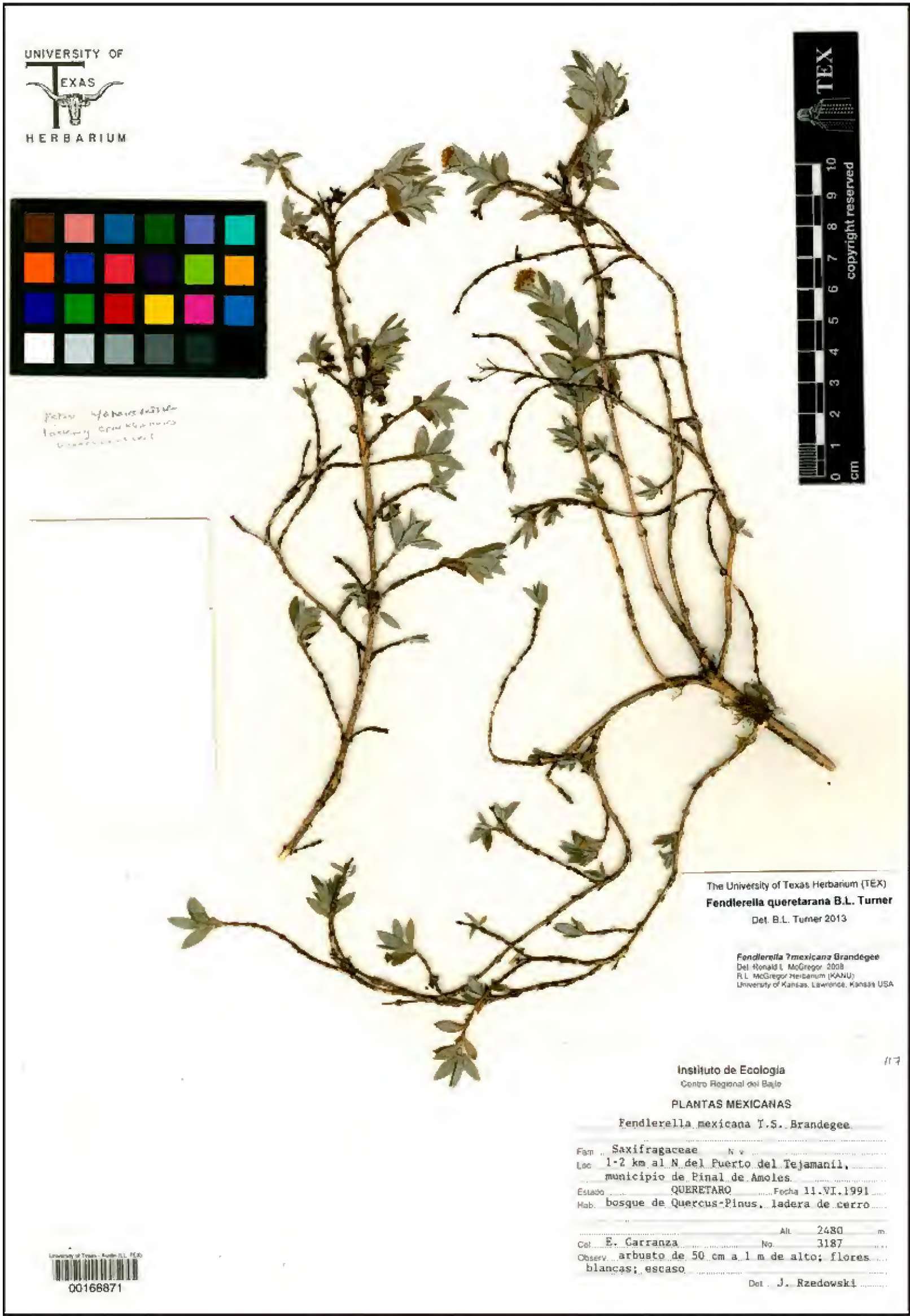


Fig. 1. *Fendlerella queretarana* (Holotype: TEX)

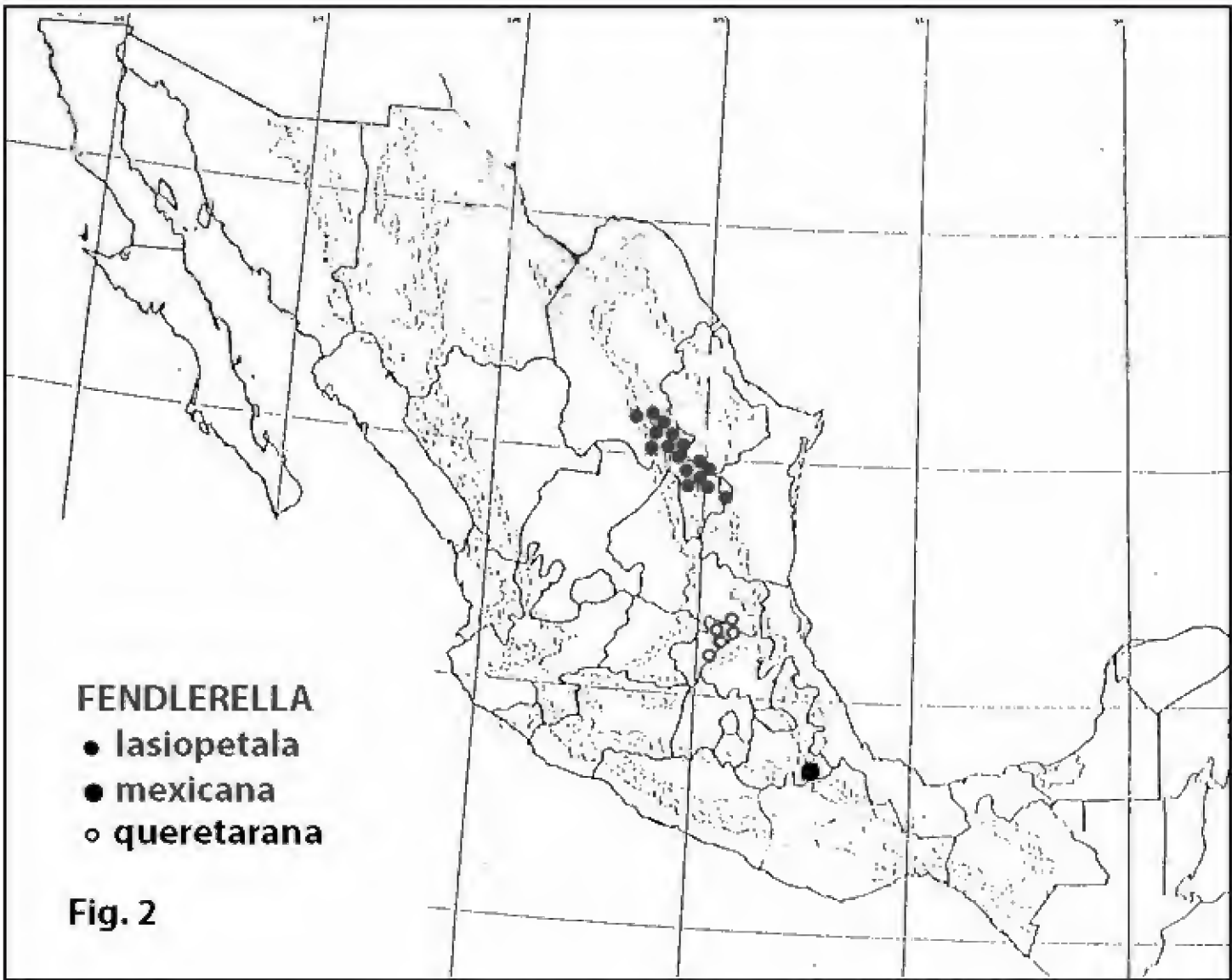


Fig. 2. Distribution of *Fendlerella* spp.

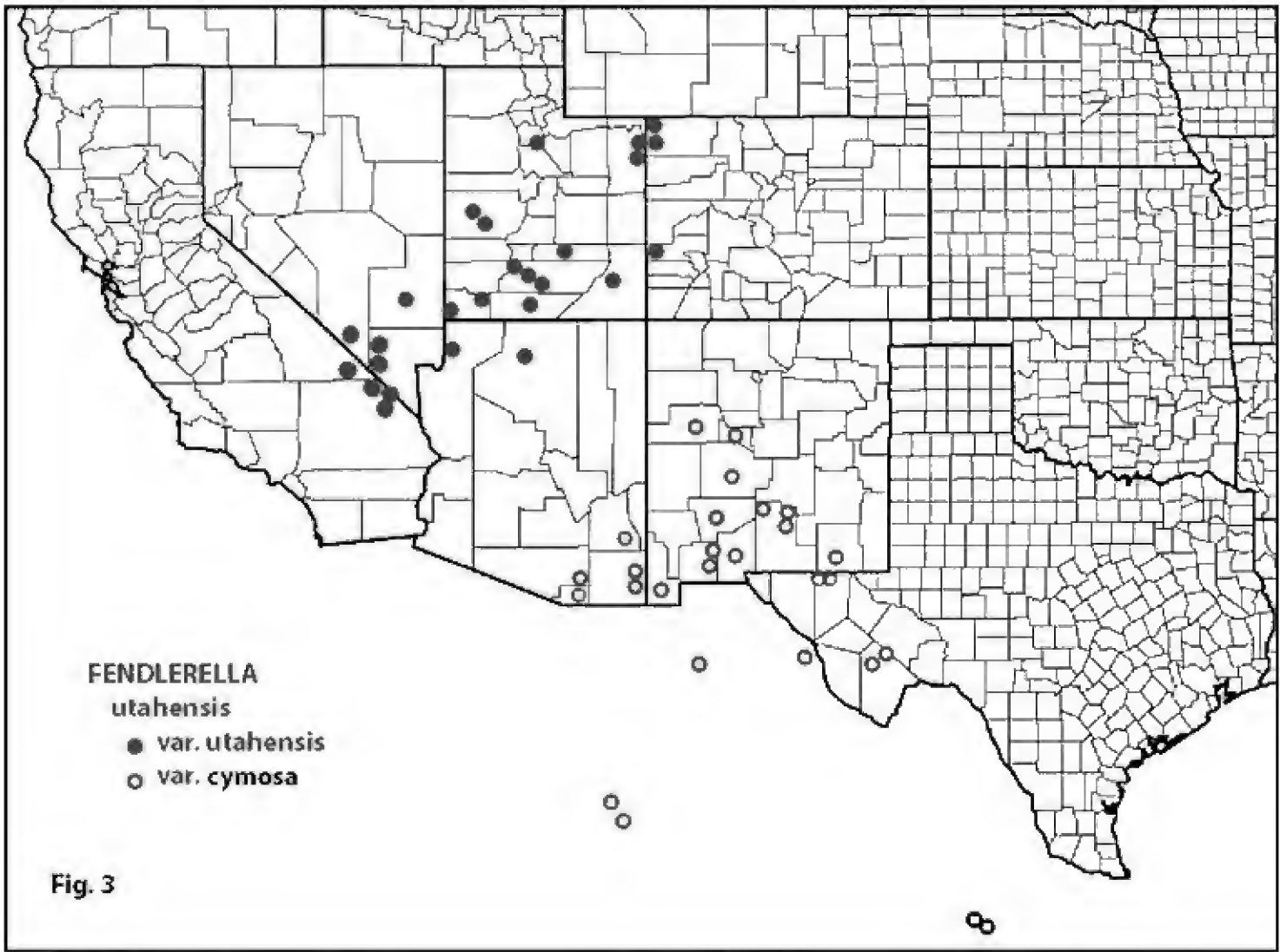


Fig. 3. Distribution of *Fendlerella utahensis*.

Cultivation of peyote: a logical and practical solution to the problem of decreased availability**Martin Terry**

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ABSTRACT

The progress toward and impediments to legally protected cultivation of *Lophophora williamsii*, commonly known as peyote, are elucidated. Recent increases in the ceremonial and medicinal consumption of peyote are inferred from published data and personal observations of the authors. The conservation-based rationale for peyote cultivation is that the predictable shift in the primary mode of production from the current unsustainable harvesting of wild peyote in habitat to regulated cultivation of peyote, either in situ or under glass, would provide alternative supplies of peyote for current and future use by the Native American Church. Such a change in the principal peyote production system from wild-harvesting to cultivation would logically reduce the harvesting pressure on the peyote populations that survive the intense overharvesting inherent in the present system. We summarize current and evolving aspects of the regulatory environment and emerging perceptions regarding the need for U.S. federal regulations that would provide legal certainty for individuals involved in the adoption of cultivation of culturally acceptable peyote on an economically viable commercial scale.

Published on-line www.phytologia.org *Phytologia* 95(4): 314-320 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: *Lophophora williamsii*, cactus conservation, sustainable production, federal regulations, Native American Church, peyote ceremonial use, peyote medicinal use, pomadas de peyote.

In this paper our objective is to provide a multidimensional snapshot of the interrelated cultural, regulatory, economic and biological factors that currently affect individuals in the U.S. who are considering embarking upon the long-term enterprise of legal cultivation of *Lophophora williamsii* (Lem. ex Salm-Dyck) J.M. Coult. (Cactaceae), hereinafter referred to as peyote. Our standing in these matters stems from our research on the conservation biology—and, inevitably, the economic botany—of peyote (Terry 2008a–c; Hulsey et al. 2011; Terry et al. 2011, 2012; Kalam et al. 2013); additionally, many of the facts and ideas presented here are based on our own personal observations.

For the sake of economy of space and the specificity of our subject, we are intentionally omitting here vast amounts of the important background information on peyote, which would include the spiritual, medicinal, and other cultural uses of this remarkable plant. For general background on such topics we would refer the reader to previously published works (e.g., Schultes 1938; La Barre 1975; Stewart 1987; Anderson 1996; Trout 1999). At this point we must illuminate an irony: There are over a thousand publications on the broad subject of peyote *consumption*. But there is not a single publication devoted to peyote *production* on an industrial scale, for the purpose of increasing the availability of the plant for the myriad of known cultural uses. In light of the alarming rate of decimation of the wild peyote populations—which are currently the *only* sources of peyote for human consumption—alternative sources

of peyote for human use are urgently required. Cultivation is the most obvious and the most readily achievable means of alternative production of peyote. Our hope is that the widespread adoption of responsible peyote cultivation by cultural groups that benefit from the use of peyote, particularly the highly decentralized, geographically scattered, heterogeneous set of entities known collectively as the Native American Church (NAC), will offer the wild populations of peyote some degree of respite from the pressures of intense overharvesting to which they are currently subject. That would allow the recovery of the wild populations, both in terms of population size and in terms of average individual size within the populations (Terry et al. 2012). Cultivation would also serve as a source of seeds of known geographic origin and juvenile plants derived from such seed, which would be suitable for augmentation of depleted populations and reestablishment of populations at sites where peyote was known to occur historically but is currently considered to be extinct.

DIMINISHING AVAILABILITY OF PEYOTE

Almost no one was thinking seriously about the need for cultivation to supplement the supply of peyote in 1970, as peyote was still relatively abundant in habitat and peyote harvesters were relatively few—even including the amateur Hippie harvesters who invaded the peyote habitat in the Texas Borderlands in the late 1960's, seeking peyote as a means of consciousness expansion or spiritual revelation as described by Huxley (1954). To be exact, discussion of peyote scarcity in the literature did not begin until George Morgan sounded the alarm—based more on prescience than on actual data—in his doctoral dissertation (1976). But as time went on and the decline of natural populations of peyote in the U.S. became more clearly and widely perceived, Morgan's voice was joined by those of cactus botanists such as Anderson (1995), Powell and Weedin (2004), and Terry (2008a–c), echoed by a plethora of articles in the popular press (e.g., Cobb 2008, de Córdoba 2004, Franks 2007, Gator 2007, Moreno 2005, Olsson 2001, Robledo 2006, Sahagun 1994, Weissert 2010) lamenting the shortage of harvestable peyote. The decline of accessible populations of mature plants suitable for harvesting for ceremonial use by the NAC was compounded by an explosive growth in the membership of the NAC accompanying the passage of the American Indian Religious Freedom Act (AIRFA) Amendments of 1994 (Prue 2013). Ironically, while certain groups within the NAC are acutely aware of the current reduced availability of peyote as the sacrament for NAC ceremonial use (MT, pers. obs.), a small but recent survey found that none of the rank-and-file NAC members interviewed was aware of any shortage of peyote (Williams 2012). Interestingly, all of the survey interviewees who were aware of a shortage were either members of the NAC leadership or a licensed peyote distributor.

An important clarification is that peyote is not universally and consistently scarce throughout its geographic distribution. There are numerous populations that are perfectly healthy, as determined by a high frequency of full-sized adult plants (as well as juveniles), but such populations in the U.S. are located either behind locked gates and high fences that are patrolled to discourage trespassing, or in such remote or physically inaccessible locations that the peyote there is effectively protected from harvesting by sheer distance and/or difficult terrain. Where peyote is scarce is precisely where the peyote harvesters have easy access (MT & KT, pers. obs.), which is a problem whose adverse effects fall most directly on the NAC.

The level of threat from other potential impacts on wild populations of peyote also needs consideration. Loss of habitat through land development is the most significant element (Anderson 1995) but also the most difficult to control. Peyote has many medicinal uses at the folk level, among both Indians and ordinary rural Mexicans. Throughout the geographic range of peyote and beyond, the home use of tinctures of crushed fresh peyote in isopropyl alcohol as a topical treatment for rheumatic pains associated with muscular fatigue is ubiquitous (Terry 2008b). That home remedy is now being produced commercially by what appears to be a cottage pharmaceutical industry with online distribution of ostensibly peyote-based products called *pomadas de peyote* (Fig. 1). Analytical work is in progress to

determine whether such products actually contain peyote, and if so, how much (MT, pers. obs.). Sales of such products, which are sold online and in open markets in the streets of Mexican cities, are difficult to quantify. If the sales reach a level compatible with industrial-scale production of the *pomadas*, that would constitute another major cause of unsustainable harvesting of peyote from wild populations.

It is worth noting that all of the threats to native populations of peyote, from agricultural practices and urban sprawl to harvesting for religious use or medicinal use, are additive and appear to be increasing wherever peyote is not actively protected from such threats.

IMPORTATION OF PEYOTE AS A “QUICK FIX” FOR REDUCED AVAILABILITY: AN EXPENSIVE RED HERRING

Leaders of the Native American Church of North America (NACNA), facing what they perceive as a severe reduction in availability of peyote for consumption as the irreplaceable sacrament in the religious ceremonies of their membership, have been continually seeking solutions to the U.S. peyote shortage since the late 1970's, mainly in the form of unsuccessful attempts to obtain concurrent regulatory approval from the Mexican government and the U.S. government for the importation of peyote from Mexico into the U.S. (Emerson Jackson, pers. comm.; MT, pers. obs.). From our perspective, the high regulatory hurdles which have prevented success in this exercise are located on the Mexican side of the Border, embedded in the regulations and policies of three quite separate agencies:

(1) Controlled substances (including peyote) are regulated by the *Departamento de Estupefacientes y Psicotrópicos*, the Department of Stupefacients and Psychotropics (DSP). In our first-hand institutional experience, the DSP was not at all comfortable with the idea of issuing an export permit (to MT) for even gram quantities of peyote for scientific research, whereas, in contrast, the U.S. Drug Enforcement Administration (DEA) issued MT an import permit for 200 g of Mexican peyote for population genetic research with apparent ease (Permit No. 3289, 5 Nov 2002). We prefer not to contemplate the apoplexy that could have been engendered in the DSP by the NACNA's proposal that the Mexican government allow the export of **ton** quantities of peyote from Mexico to the U.S. for ingestion.

(2) Endangered and threatened species (which means all Mexican species of the family Cactaceae, including peyote) are regulated by the *Secretaría de Medio Ambiente y Recursos Naturales* (SEMARNAT), the Secretariat of Environment and Natural Resources, one of whose duties is to promulgate and enforce national regulations to implement the International Convention on Trade in Endangered Species (CITES), of which both Mexico and the U.S. are signatories. In accordance with CITES, there is a Mexican federal regulation listing all known endangered and threatened species of Mexican cacti and their classifications in regard to their risk of extinction and priority of regulatory attention, originally published as NOM-059-SEMARNAT-2002, and most recently updated as NOM-059-SEMARNAT-2010. In this regulation, the species *Lophophora williamsii* is placed in a risk category that contains very few species; that category prescribes that the species is “*subjeta a protección especial*” (subject to special protection). This classification of peyote suggests that it is not considered endangered by SEMARNAT, but that it is considered to be at greater risk than most of the non-endangered species in the Cactaceae. That classification by SEMARNAT would surely raise red flags of regulatory caution in response to any proposal from a foreign entity, such as the NACNA, to increase the rate of exploitation of Mexican populations of peyote for the sole purpose of exportation to the U.S.

(3) Indigenous Mexican peoples who traditionally use peyote would be expected to have the support of the *Comisión Nacional para el Desarrollo de los Pueblos Indígenas* (CDI), the National Commission for the Development of the Indigenous Peoples. Much depends on whether the Mexican indigenous groups are sufficiently well connected with the government to voice their concerns to CDI, and whether they perceive that the wild-harvesting and export of massive quantities of peyote from Mexican territory

may jeopardize their own future access to peyote. But it is at least reasonable to assume that the CDI would place a higher priority on maintaining the availability of peyote to Mexican indigenous peoples than on solving the problem of reduced availability of peyote for non-Mexican indigenous groups who face a dwindling domestic supply from their own peyote populations.

The preceding considerations are historically important because of the negative bearing they have had on any considered efforts to explore cultivation of peyote in the U.S. The seductive goal of persuading the Mexican government to allow the exportation of Mexican peyote to the U.S. for NACNA ceremonial use has been a perennial red herring in NACNA politics. Time will be required for (1) establishing the U.S. regulatory parameters for cultivation and (2) climbing the technical and financial learning curve to establish large-scale cultivation of peyote. Both will need to occur before cultivation can replace the overharvested South Texas populations as the primary source of sacramental peyote for the NAC.

THE LEGAL AND REGULATORY ENVIRONMENT IN THE U.S.

The challenges inherent in initiating peyote cultivation in the U.S. were exacerbated by the enactment of the Controlled Substances Act (U.S. Congress 1970) and the DEA regulations that were subsequently adopted in the iterative process of implementing the legislation. The crux of the problem was that the primary purpose of the Controlled Substances Act (CSA) was to control the use of drugs, and in the case of the substances relegated to Schedule 1, to eliminate such use if possible. Schedule 1 is the CSA drug category containing what may be considered the most stringently forbidden drugs, defined as those with “no currently accepted medical use in the United States, a lack of accepted safety for use under medical supervision, and a high potential for abuse” (U.S. Congress 1970). For better or for worse, peyote was swept into Schedule 1 along with hallucinogens such as LSD. That meant that the policy makers at DEA were primarily tasked with making peyote **difficult** to obtain — **not** with ensuring that the Native American Church (whose members were exempted from the prohibition on peyote use, per 21 C.F.R. § 1307.31) would be able to obtain **enough** peyote as time went on. Indeed, the most direct translation of the horticultural phrase “cultivation of peyote” into the regulatory language of Title 21 of the Code of Federal Regulations is “manufacturing [a] controlled substance” (Drug Enforcement Administration 2013).

In 1994, Congress created more helpful language, specifically in reference to the cultivation of peyote. The American Indian Religious Freedom Act (AIRFA) Amendments of 1994 clarified that the law “does not prohibit such reasonable regulation and registration of those persons who cultivate, harvest, or distribute peyote as may be consistent with the purposes of this Act.” The legislative language also made it clear that among “the purposes of this Act” was that of providing “adequate and clear legal protection for the religious use of peyote by Indians.” So there is no impediment to the cultivation of peyote at the legislative level. The only component lacking is at the regulatory level. That is where DEA regulations are needed to create the appropriate regulatory structure that will provide “adequate and clear protection for the religious use of peyote by Indians.” In order to comply with the intent of this language of the statute, it is incumbent upon DEA to take into account the fact that “the religious use of peyote by Indians” is compromised by the reduced availability of peyote. That fact leads directly to the logical conclusion that the reduced supplies of wild-harvested peyote must be augmented with peyote produced by regulated cultivation undertaken by registered persons.

Then why has no one petitioned DEA to promulgate the appropriate regulations, negotiated between interested NAC members and DEA, to enable cultivation of peyote in accordance with AIRFA 1994? Well, in fact, someone has, but the negotiations were not productive (MT & KT, pers. obs.). When the next petition for regulatory relief in the form of peyote cultivation is submitted to DEA, a concerted and timely effort will be required of both DEA and NAC if negotiations aimed at the time-sensitive promulgation of the needed regulations are to be successful.

Another possibility is that, if the DEA is too slow to respond to NAC needs expressed in petitions for regulated cultivation of peyote, the populations of peyote in the U.S. will become so decimated that the U.S. Fish and Wildlife Service (USFWS) will be legally obligated to step in to regulate peyote as a threatened species under the Endangered Species Act (ESA) (U.S. Congress, 1973). In that event, the ESA calls for interagency consultations with other interested government agencies, and that would mean that the DEA would be required to consult with the USFWS, and it does not take much imagination to predict that an alternative to the current system of wild-harvesting of peyote—viz., cultivation—would be at the top of the agenda.

Yet another possibility is that DEA will delay the process of promulgating the regulations until it makes itself irrelevant to the development of cultivation of peyote by the NAC. One major tribe has already taken the decision to cultivate peyote on tribal land — that is to say, on land which is governed by the tribe as a sovereign nation, and which is largely protected from U.S. regulatory interference (particularly in matters of Indian religion) by the veil of national sovereignty (MT, pers. obs.).

Cultivation seems to be an inevitable undertaking in the future of the NAC if they envision a long-term future for the religious use of peyote. Delaying implementation of cultivation compounds their challenges due to the lag time involved prior to the first large-scale harvest in a sustainable production stream. We estimate that the developmental lag time to full-scale production of culturally acceptable peyote will be on the order of 10 years. The sooner that task can begin, the simpler the future will be for everyone involved, from the level of the average NAC member participating in ceremony to the regulator involved with creating an acceptable regulatory framework.

ACKNOWLEDGMENTS

We wish to thank A.M. Powell and Billie Turner for reviewing the manuscript and offering helpful comments. Kevin Feeney provided timely help with the legal citations. We also thank Sul Ross State University for a Research Enhancement grant that funded some of the field research that lead to the writing of this article. The Alvin A. and Roberta E. Klein Foundation supported travel to professional meetings where the ideas in this paper were developed.

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Figure 1. A *pomada de peyote* (commercial “peyote” ointment for topical application), as sold openly in the public markets of Mexico. It remains to be determined whether such products actually contain any components of *L. williamsii*.

Taxonomy of *Physaria purpurea* (Brassicaceae)

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ABSTRACT

Physaria purpurea (A.Gray) O’Kane & Al-Shehbaz is recognized as a widespread variable species without infraspecific taxa, much as accorded the taxon by Rollins (1993) in his last appraisal of the species. Distribution maps of the reputed infraspecific categories are provided, along with an overall reappraisal of the complex. Published on-line www.phytologia.org *Phytologia* 95(4): 321-323 (Nov. 1, 2013). ISSN 030319430

KEY WORDS: Brassicaceae, *Lesquerella*, *L. purpurea*, *Physaria*, *P. purpurea*, Texas, USA, Mexico

Rollins and Shaw (1973) provided an excellent taxonomic treatment of the species, *Lesquerella purpurea* (A. Gray) Wats., recognizing the taxon as having two infraspecific taxa, subsp. *purpurea* and subsp. *foliosa* (Rollins) Rollins & Shaw, according to the authors, these presumably intergrading in Trans-Pecos, Texas. The characters used to identify the infraspecific taxa were given as follows (this largely abstracted from their account):

1. Stems sparsely leaved and extending well beyond the basal leaves; petals ca 7-12 mm long, having a distinct blade and claw; trichomes 0.3-0.4 mm in diameter.....subsp. **purpurea**
1. Stems but little extended beyond the basal leaves, and/or the stems leafy and branched in the upper half; petals 4-8 mm long, and often w/o distinct blades and claws; trichomes 0.4 mm or more in diameter.....subsp. **foliosa**

The type of subsp. **purpurea**, according to Rollins, is a Wright collection from El Paso Co., Texas; the type of subsp. **foliosa** is a Johnston & Mueller collection from the Sierra San Carlos, a small mountain range just south of the Big Bend region of Texas.

Early on, I mapped the infraspecific taxa, as shown in Fig. 1, largely from the sites posited by Rollins and Shaw. Rollins (1993) subsequently, without explanation, treated *Lesquerella purpurea* as lacking infraspecific units. Nevertheless I preferred to accept the earlier interpretation of Rollins and Shaw, and when transferring these to the genus *Physaria*, following the work of O’Kane and Al-Shehbaz (2002), I inexcusably misstated (Turner 2004) that the latter workers treated *P. purpurea* var. *foliosa* as a subspecies, which they did not.

To complicate things further, in my Atlas of the Vascular Plants of Texas (Turner et al., 2003) I accepted the treatment of Rollins (1993), in which no infraspecific taxa were recognized, this after examining newly assembled plants from both the USA and Mexico. In short, I now accept *Physaria purpurea* (A.Gray) O’Kane & Al-Shehbaz as an exceedingly variable taxon occurring throughout the south-central USA into north-central Mexico, from Coconino Co., Ariz. to as far south as San Luis Potosi, as shown in Fig. 2, much as did Rollins (1993) in his ultimate appraisal of the complex, this without explanation.

ACKNOWLEDGEMENTS

My editorial colleague, Jana Kos, proofread the paper, providing helpful comments. The dot maps are based upon specimens cited by Rollins and Shaw (1973), and collections on file at SRSC and LL-TEX.

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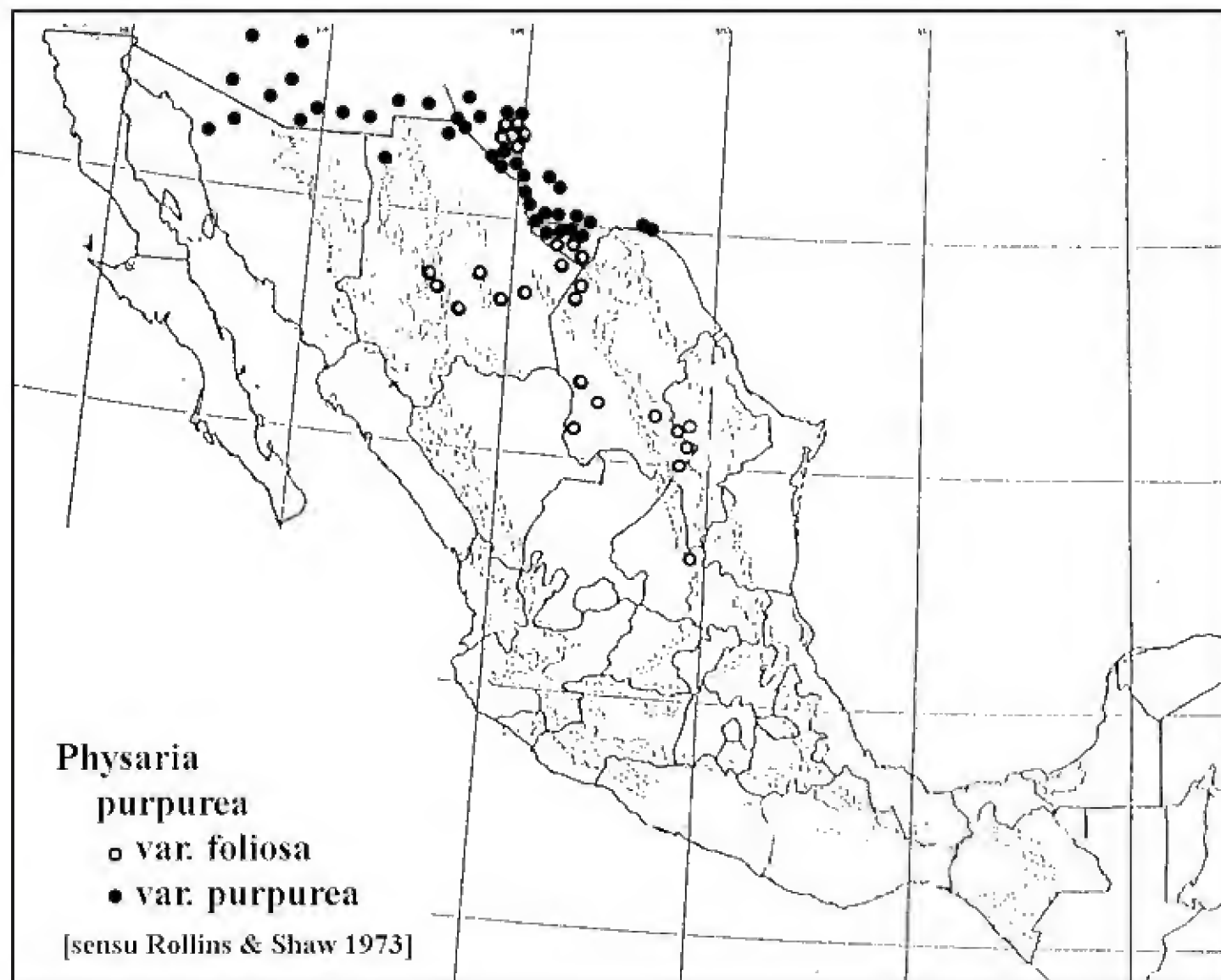


Fig. 1. Distribution of infraspecific categories of *Physaria purpurea*, sensu Rollins and Shaw (1973).

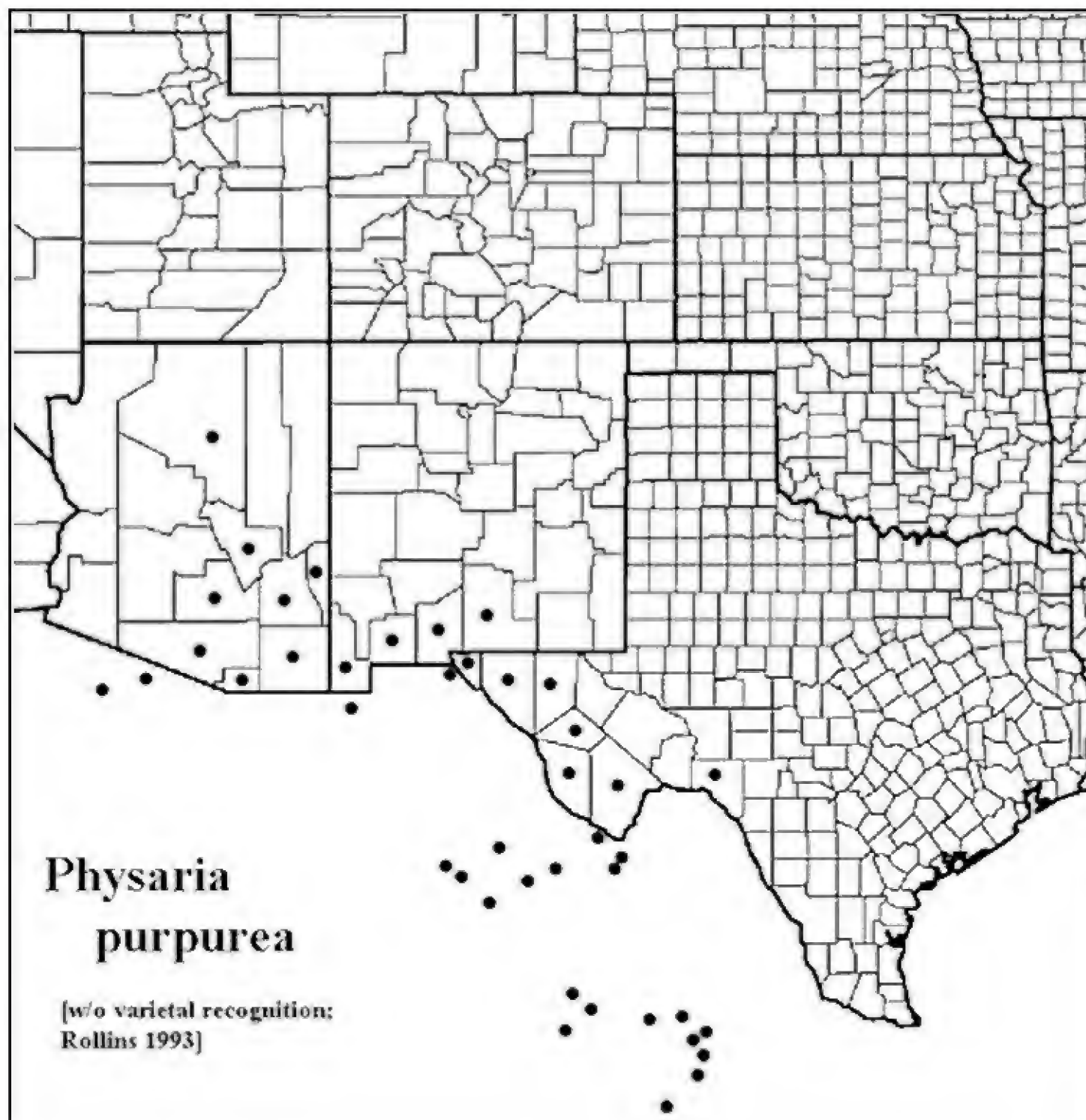


Fig. 2. Distribution of an integrated *Physaria purpurea* (sensu Rollins 1993).